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# PROGRESSIVE CHANGE IN THE INSECT POPULATION OF FORESTS SINCE THE EARLY TERTIARY[1]

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ONE afternoon some twenty years ago during a winter sojourn in northern Florida the writer was collecting insects in a pine forest given over to the production of turpentine. Following the usual practice, the bark and sapwood of each tree had been cut away for a distance on one side and a pot hung at the bottom to catch the resin. The scarred trunks of the disfigured trees were reeking with oozing resin, and here and there insects of various kinds were imbedded where they had been trapped by the sticky exudation. Mrs. Brues was with me at the time and, knowing that I was interested in amber insects, she suggested that we make a collection of these insects from the turpentine trees to compare with the fossil fauna of the amber. We could see several interesting possibilities in such an undertaking and proceeded forthwith to gather the material. The specimens were transferred to vials of alcohol and after a time we had amassed a considerable and very miscellaneous assortment which assumed a much better appearance after the alcohol had dissolved the envelope of resin.

The matter had to be held in abeyance for many years, until I had been able to devote some time to a study of one small part of the insect life of amber. It then appeared that the Florida collection was too small to be of

[1] From the Entomological Laboratory, Harvard University.

any considerable value, and another more extensive one was then made in what appears to be a much more suitable region. This I shall deal with in a few moments.

Amber is fossilized and greatly hardened resin. It occurs in lumps or nodules of various sizes, practically in the same form that it first developed as an exudation of sticky resin on the trunks of pines in the amber forests. These pieces are often of quite considerable size, frequently weighing several pounds, and represent the only known remains of magnificent forests that flourished in northern Europe in upper Eocene times. As the fresh resin exuded, its sticky surface trapped innumerable small flying and crawling insects, together with other objects that fell or were blown against it, to be engulfed and permanently preserved just as we now mount small insects in Canada balsam. Many fragments of the plants that grew in the amber forests are thus preserved also, enabling us to gain a far clearer idea of the insects and associated plants than is possible in the case of any other extinct insect-fauna.

As to the flora of the amber forests we are able to form quite a complete picture from the bits of plant remains that are preserved in amber nodules. It is believed that the most abundant tree was a true pine, *Pinus succinifera* Göppert, from which the amber was very probably derived, at least in great part. However, this tree was associated with an extensive and varied sylvan flora which included other conifers of the living genera Sequoia, Taxodium, Picea, Thuja and a somewhat dubious form known as Pinites. Among deciduous trees there were abundant oaks of numerous species, as well as chestnuts, beeches, bays (*Myrica*), magnolias, cinnamons (*Cinnamomum*) and several palms. The composition of the arborescent flora is therefore not exactly reproduced anywhere at the present time, but is quite similar to that of North America. In the eastern United States most of these trees have living relatives, several types restricted to the south, but the most abundant ones now more com-

mon farther north. Some have pointed out the great similarity of the present flora of Florida, particularly on account of the palmetto palm, magnolia and bay-tree, but the agreement here seems no greater than in New England, on account of the spruces and beeches, and certainly the terrain of the amber forests, as we know it from the caddis-fly fauna, was not that of the flat coastal plain. Undoubtedly the climate was much milder than the one which we now enjoy in the northeastern states, and the forests were clearly comparable with our own, as they existed several centuries ago before their exploitation was begun by civilized man.

The history of Baltic amber after its deposition on the tree-trunks has been quite accurately traced. The amber forests occupied an extensive area in Europe north of the Baltic sea. The amber remained after the death and disintegration of the trees which had produced it, and some is now found in a deposit of so-called "blue earth" which represents the amber-bearing strata of upper Eocene age. Here the amber was apparently carried by streams or rivers and thus accumulated. Much amber is found also in certain stratified sands of Miocene age, where it has been redeposited, commonly in rich pockets. Still later, smaller amounts were distributed by inland ice at the time of glaciation in the north. Most of the original deposits now lie below the surface of the Baltic Sea, where they can not be worked systematically, but from time to time through the agency of severe storms pieces are dislodged. As the amber is lighter than water it rises and drifts ashore where it may be recovered. On occasions as much as a ton or more has been collected on the shores of the Baltic after a stormy night. For a study of the inclusions the amber is commonly sawed into rectangular blocks, each containing an individual specimen and the surface polished to permit observation through the transparent matrix. Much of the amber is not transparent, and as the included insects, trash and other objects are scattered at random, the discovery and prepara-

tion of good specimens is a very slow and laborious process. Thus prepared, examination under the microscope is readily possible, even under high powers, except where flaws in the amber, clouding of the inclusions or superposition of parts interfere with clear vision. Ordinarily the more minute specimens are better preserved than the larger ones, as there is less clouding from water vapor or mould, and they are less apt to have become broken during the process of submergence in the resin.

Thus nature has preserved a very considerable fragment of the rich and varied forest fauna that flourished in Northern Europe some 40,000,000 years ago.

For a number of years I have been interested in a small part of this insect fauna of Baltic amber, comprising a series of families of parasitic species belonging to the order Hymenoptera. After some time spent in sorting, classifying and describing the new genera and species found in the amber I gradually became aware of certain apparent differences in the representation and diversity of the several groups in the fossil amber fauna and that which survives it at the present day. At first blush these seemed to indicate progressive changes in the numerical abundance of certain groups of insects that are well represented in both the amber and recent fauna.

Such changes are certainly to be expected, for we have abundant evidence in practically all groups of animals and plants that evolutionary change has entailed the appearance of numerous particular groups that have waxed abundant and later waned, often to extinction. Due to the limitations of the case, however, the paleontologist must ordinarily concern himself primarily with the morphological aspect of such changes as they relate to the appearance of types, families or genera and their diversification into lesser types and species. Aside from comparative anatomical studies, speculative deductions and the application of certain principles, our entire knowledge of the phylogeny of animals and plants rests upon this

very firm foundation of observed factual evidence. One aspect of the composition of fossil faunas lends itself to observation in only a very crude and incomplete way, since we can not estimate with any degree of accuracy the proportionate numerical abundance or dominance of particular groups or of individual species. This is, of course, due to the fact that certain happy combinations of circumstances are requisite for the preservation of organisms as fossils. Moreover, we know that such combinations have by no means necessarily been similar on different occasions. Most deposits that contain insect remains have been laid down in water, but we are unable to do more than surmise what portions of the prevalent insect fauna have been preserved in any particular bed. We can piece together little evidence concerning the terrain or the flora of the immediate vicinity, nor are we able to say what part transport by wind or carriage by streams may have played in assembling the population sample that we may be fortunate enough to unearth after the lapse of long periods. If certain groups or species are well represented by specimens we know that they must have formed a considerable part of the insect population, but if others are less numerous it is quite probable that they may have for some reason escaped entombment and even if some are absent we may easily find a good reason for their failure to chance upon that spot at the appropriate time. The great preponderance of winged ants in the fauna of the Miocene shales of Florissant is very probably due to storms of volcanic dust which overwhelmed them in flight, while the contrasting abundance of wingless worker ants in the Baltic amber is no doubt due to the more peaceful method by which they were trapped. Thus neither deposit can afford data for an accurate census of the sexual and worker phases of ants during tertiary times.

To return again to the collection of amber insects mentioned previously, it will be seen that this affords an opportunity to compare with considerable accuracy the

Oligocene with the recent insect fauna. We know that the amber fossils are species that frequented the trunks of pine trees in areas of mixed forest quite similar to those now present in the northeastern United States. Furthermore, the genera of insects known from the amber show that this Eocene fauna was more similar to that of the nearctic region at the present time than to the living fauna of any other single part of the world. From his studies on the Trichoptera or caddis-flies of the amber, Ulmer (1912) has reached conclusions concerning the topography of the amber forests which supplement and confirm those drawn from a knowledge of the types of trees present there. The trees indicate a partly mountainous country, and the caddis-flies seem to demonstrate without question the existence of rushing mountain streams, slowly flowing brooks and quiet ponds. Since many genera of caddis-flies are closely restricted in habitat, Ulmer concludes from the representation of modern genera in amber that the number of genera and species requiring mountain streams greatly exceeded the other types, although the latter were present in sufficient abundance to show clearly the existence of the static or quiet water habitat.

These considerations indicate that ecological conditions in the amber forests are rather closely reproduced in the hilly forested areas of New England at the present time, and as already indicated the flora and insect fauna were quite similar to those now existing in that region.

It seemed, therefore, that this region offered a particularly good opportunity to compare in detail the numerical abundance of specific groups of insects as they are preserved in amber with the present forest fauna. This required that a census of forest insects be taken under conditions closely approximating those which led to the formation of the amber inclusions.

In order to collect a closely similar sample of the insect population we made use of the well-known tanglefoot fly paper, which is easily obtainable and proved to be

eminently suited to the purpose. Tanglefoot simulates closely in its sticky surface fresh exuding resin; the sheets may be readily tacked to the trunks of trees and later removed for inspection, so that they serve to collect just that portion of the forest fauna directly comparable with the one found in the amber. It may be asked what differences may be expected when the piney odor of fresh resin is replaced by that of the tanglefoot. There is every reason to believe that odor has little influence in trapping the insects, since most of the species in amber are very evidently in no way directly associated with resin or with the trees which produced it. The trapping of most specimens is undoubtedly by pure accident, *e.g.*, the various Diptera which represent over 50 per cent. of the whole population both in the amber and tanglefoot collections. Even in the case of bark beetles of the family Scolytidae, where some of the amber species must have been associated with the resin-producing trees, the representation of this family is extremely low and quite similar to the scarcity noted in the tanglefoot collection. I believe therefore that no considerable differences in sampling have been introduced by the use of tanglefoot in place of resin.

The localities selected for the tanglefoot collections were all in the township of Petersham, which lies in northern Massachusetts, at an elevation of from 800 to 1,100 feet above sea-level. As nearly as possible areas of well-matured (including some nearly primeval) forest were chosen where there was either a predominance of conifers (white pine and hemlock) or a mixed growth including also considerable beech, oak, maple and birch. The terrain included areas of well-drained higher land, damper spots, open spaces and the borders of both swiftly and slowly flowing brooks. Thus, as nearly as could be judged, the localities which scattered for some miles through the Harvard Forest and on land adjoining our own summer home, represented the several ecological conditions known to have existed in the amber forests and in about the same proportion.

Collections were made in the several localities during the course of the entire summer of 1930 from early May till late September. Sheets of the tanglefoot fly paper were fastened by tacks at each corner to the trunks of trees at different heights that could be conveniently reached from the ground. After a few days numerous insects were caught and the surface of the tanglefoot became less sticky. The papers were than removed, each carefully curled into a cylinder with the sticky surface inside, caught thus with pins and brought into the laboratory. The immersion of each cylinder in a large jar of 95 per cent. alcohol for a few minutes dissolves the matrix and allows the specimens to float free. In spite of this treatment the insects are in very good condition and after removal to vials of clean alcohol may later be conveniently sorted in watch glasses under the microscope. During the course of our work both Mrs. Brues and myself commented frequently and vehemently on the great abundance of blackflies, mosquitoes and biting flies (Chrysops) in the forest, and gloated in prospect over the swarms of these pests that would be engulfed in the tanglefoot before the end of the season. Subsequently, when the material was sorted we were pained to learn that only 26 mosquitoes, three Chrysops and 18 Simulium appeared in the collection of 22,938 specimens, more than 21,000 of which were insects. This is an extreme case, but shows very clearly that the wielding of a net or some other method of collecting in the forest would have produced a very differently selected sample of the insect population, which could not have served for adequate comparison with the amber.

The census of the tanglefoot material has occupied some time and would have been delayed still longer had it not been for very considerable aid from Mrs. Brues and my secretary, Mrs. O'Connor, who did much of the tedious preliminary sorting. As may be seen from the accompanying tabular list, the specimens belonging to some of the more abundant orders have been sorted to

families; other groups, such as the Corrodentia and Trichoptera, have not been thus divided, as my knowledge of the families was not sufficient to insure accuracy in placing broken or otherwise defective specimens.

CENSUS OF TANGLEFOOT POPULATION

| | | | |
|---|---|---|---|
| Collembola | 60 | Psychodidae | 165 |
| Orthoptera | | Chironomidae | 652 |
| Stenopelmatidae | 11 | Culicidae | 26 |
| Acrididae | 1 | Simuliidae | 18 |
| Thysanoptera | 6 | Bibionidae | 3 |
| Corrodentia | 209 | Mycetophiloidea | 2,648 |
| Mallophaga | 1 | Cecidomyiidae | 141 |
| Homoptera | | Rhagionidae | 906 |
| Membracidae | 2 | Tabanidae | 3 |
| Cercopidae | 106 | Therevidae | 1 |
| Jassidae | 1,685 | Asilidae | 4 |
| Cicadellidae | 472 | Empididae | 559 |
| Aphididae | 81 | Dolichopodidae | 3,070 |
| Chermidae | 11 | Phoridae | 6,443 |
| Hemiptera | | Platypezidae | 1 |
| Tingididae | 18 | Pipunculidae | 21 |
| Nabidae | 3 | Syrphidae | 99 |
| Miridae | 51 | Muscoidea | |
| Odonata | 1 | Thecostomata | 287 |
| (Zygopteran nymph) | | Haplostomata | 1,195 |
| Plectoptera | 35 | Coleoptera[2] | |
| Plecoptera | 124 | Carabidae | 8 |
| (incl. 2 nymphal cases) | | Silphidae | 14 |
| Neuroptera | | Scydmaenidae | 12 |
| Hemerobiidae | 7 | Staphylinidae | 31 |
| (incl. 2 larvae) | | Pselaphidae | 4 |
| Coniopterygidae | 5 | Scaphidiidae | 2 |
| Mecoptera | | Cucujidae | 1 |
| Meropidae | 2 | Lycidae | 3 |
| Panorpidae | 37 | Lampyridae | 43 |
| Trichoptera | 332 | Cantharidae | 87 |
| Lepidoptera | | Melyridae | 1 |
| Noctuidae | 4 | Cleridae | 1 |
| Geometridae | 6 | Mordellidae | 101 |
| "Micros" | 153 | Meloidae | 1 |
| Nymphalidae | 1 | Pyrochroidae | 1 |
| Caterpillars | 12 | Oedemeridae | 1 |
| Diptera | | Elateridae | 52 |
| Tipuloidea | 260 | Eucnemidae | 1 |

[2] I am greatly indebted to Dr. P. J. Darlington, of the Museum of Comparative Zoology, who checked the series of Coleoptera and examined all doubtful specimens in this order.

| | |
|---|---|
| Throscidae | 74 |
| Dryopidae | 1 |
| Dascillidae | 7 |
| Helodidae | 25 |
| Dermestidae | 6 |
| Ptinidae | 1 |
| Cryptophagidae | 1 |
| Erotylidae | 1 |
| Mycetaeidae | 1 |
| Colydiidae | 11 |
| Lathridiidae | 27 |
| Endomychidae | 2 |
| Coccinellidae | 2 |
| Alleculidae | 4 |
| Melandryidae | 26 |
| Anobiidae | 12 |
| Cerambycidae | 3 |
| Chrysomelidae | 5 |
| Curculionidae | 8 |
| Scolytidae | 3 |
| Scarabaeidae | 1 |
| Hymenoptera | |
| Xyelidae | 1 |
| Pamphiliidae | 1 |
| Tenthredinoidea | 8 |
| Braconidae | 59 |
| Alysiidae | 76 |
| Ichneumonidae | 492 |
| Chalcidoidea | 46 |
| Mymaridae | 13 |
| Roproniidae | 1 |
| Serphidae | 4 |
| Belytidae | 152 |
| Diapriidae | 23 |
| Scelionidae | 25 |
| Calliceratidae | 57 |
| Platygastridae | 114 |
| Dryinidae | 3 |
| Cynipoidea | 8 |
| Formicidae | 67 |
| (including one larva) | |
| Vespidae | 5 |
| Other wasps | 10 |
| OTHER ARTHROPODS | |
| Diplopoda | 9 |
| Chilopoda | 1 |
| Araneida | 1,045 |
| Chelonethida | 3 |
| Acarina | 13 |
| Phalangida | 179 |
| Total | 22,938 |

More than half, in fact 71.9 per cent. of the entire tanglefoot collection, are Diptera, 16,484 in all; next in abundance are Homoptera with 2,429, followed by Hymenoptera (1,165) and spiders (1,045). All other orders, together with scattering other arthropods, make up less than 8 per cent. of the collection. This must not, of course, be regarded as a sample of the entire forest population, but it is, as previously indicated, a sample on which we may rely to determine with at least some measure of accuracy what changes have taken place in the forest population during the long period that has passed since the amber was laid down.

For this purpose it will be most satisfactory to examine first two of the groups whose representation in the amber is especially well known. For one of these we have accurate and extensive data furnished by Klebs (1911). As a pioneer student of amber inclusions, Dr. Klebs

amassed an enormous collection, later bequeathed to the Geologisch-paläontologisches Institut at Königsberg. The comparative representation of the more abundant families of Coleoptera in the population of Baltic amber and in the present-day New England fauna is shown in the accompanying table (Table I). Great divergence

TABLE I

COMPARATIVE REPRESENTATION OF THE MORE ABUNDANT FAMILIES OF COLEOPTERA IN THE POPULATION OF BALTIC AMBER AND THE PRESENT-DAY NEW ENGLAND FAUNA

| | Amber | Per cent. | Recent* | Per cent. | Recent, Actual Numbers |
|---|---|---|---|---|---|
| Carabidae | 72 | 3.7 | 27 | 1.4 | 8 |
| Silphidae | 9 | 0.4 | 47 | 2.4 | 14 |
| Scydmaenidae | 28 | 1.4 | 40 | 2.0 | 12 |
| Staphylinidae | 69 | 3.5 | 103 | 5.3 | 31 |
| Pselaphidae | 24 | 1.2 | 13 | 0.7 | 4 |
| Cucujidae | 17 | 0.9 | 3 | 0.2 | 1 |
| Lampyridae | 3 | 0.1 | 143 | 7.5 | 43 |
| Cantharididae | 51 | 2.6 | 290 | 14.3 | 87 |
| Mordellidae | 115 | 5.9 | 337 | 17.3 | 101 |
| Hylophilidae | 53 | 2.7 | 0 | 0.0 | 0 |
| Anthicidae | 49 | 2.5 | 0 | 0.0 | 0 |
| Elateridae | 286 | 14.1 | 173 | 8.9 | 52 |
| Eucnemidae | 48 | 2.4 | 3 | 0.2 | 1 |
| Throscidae | 17 | 0.9 | 247 | 12.2 | 74 |
| Helodidae | 376 | 19.3 | 83 | 4.2 | 25 |
| Dermestidae | 5 | 0.3 | 20 | 1.0 | 6 |
| Cryptophagidae | 16 | 0.8 | 3 | 0.2 | 1 |
| Colydiidae | 14 | 0.7 | 37 | 1.9 | 11 |
| Lathridiidae | 41 | 2.1 | 90 | 4.8 | 27 |
| Alleculidae | 17 | 0.9 | 13 | 0.7 | 4 |
| Melandryidae | 44 | 2.3 | 87 | 4.7 | 26 |
| Anobiidae | 236 | 12.1 | 40 | 2.0 | 12 |
| Cerambycidae | 39 | 2.0 | 10 | 0.5 | 3 |
| Chrysomelidae | 30 | 1.5 | 17 | 0.9 | 5 |
| Curculionidae | 47 | 2.4 | 27 | 1.4 | 8 |
| Scolytidae | 37 | 1.9 | 10 | 0.5 | 3 |
| Totals | 1780 | 91.2 | 1863 | 95.5 | 585 |

* These numbers have been increased, each in proportion, to compare directly the two populations on the basis of equal size, *i.e.*, the figures in this column are each increased by three and one third times.

will be noticed in the abundance of certain families in each fauna and a very marked change from one fauna to the other is shown in the case of practically every family that is especially abundant either in the amber or in the tanglefoot collection. I do not believe, however, that any definite trend is indicated, such as an increasing abundance of highly specialized types. This might appear to be true, for example, with reference to the increased abundance of Mordellidae, but this is at once offset by an increase in several unquestionably primitive families like the Carabidae, Lampyridae and Cantharididae. The last two indicate, however, a much increased abundance of the more highly modified Lampyridae. It seems, however, that several much specialized families have decreased very noticeably. We may therefore gain little information from the beetles, but as I shall show in a moment they serve to supplement evidence supplied by other groups.

On another tabular chart (Table II) I have listed the several families of parasitic Hymenoptera that I myself have studied in the Baltic amber, together with the data gathered from the tanglefoot collection. An examination of these figures shows a series of consistent changes which appear to be highly significant. Among the ichneumon flies, the two dominant families, Ichneumonidae and Braconidae, which are both well represented in each fauna, have changed places. The more primitive Braconidae, which are somewhat more abundant than the Ichneumonidae in amber, have been reduced in number to about one fourth that of the Ichneumonidae. Among the Serphoidea the families Scelionidae and Platygastridae have likewise changed places. These two families are very similar, but no one can question the more primitive nature of the Scelionidae. The latter have dropped from 15.6 per cent. to 2.4 per cent., while the population of Platygastridae has increased from 1.5 per cent. to 10.6 per cent. Another family of primitive wasps, the Bethylidae, does not appear at all in the tanglefoot col-

TABLE II

COMPARATIVE REPRESENTATION OF THE FAMILIES OF PARASITIC HYMENOPTERA IN THE POPULATION OF THE BALTIC AMBER AND THE PRESENT-DAY NEW ENGLAND FAUNA

| | Amber | Per cent. | Recent* | Per cent. | Recent, Actual Numbers |
|---|---|---|---|---|---|
| Aulacidae | 4 | 0.2 | 0 | 0.0 | 0 |
| Stephanidae | 5 | 0.3 | 0 | 0.0 | 0 |
| Evaniidae | 35 | 1.9 | 0 | 0.0 | 0 |
| Megalyridae | 28 | 1.5 | 0 | 0.0 | 0 |
| Pelecinopteridae | 3 | 0.2 | 0 | 0.0 | 0 |
| Braconidae (incl. Alysiidae) | 331 | 18.2 | 227 | 12.4 | 135 |
| Ichneumonidae | 292 | 16.1 | 832 | 45.8 | 492 |
| Chalcidoidea (excl. Mymaridae) | 191 | 10.5 | 78 | 4.3 | 46 |
| Mymaridae | 26 | 1.5 | 22 | 1.2 | 13 |
| Roproniidae | 0 | 0 | 2 | 0.1 | 1 |
| Serphidae | 74 | 4.0 | 7 | 0.4 | 4 |
| Diapriidae (incl. Belytidae) | 258 | 14.2 | 296 | 16.2 | 175 |
| Scelionidae | 285 | 15.6 | 43 | 2.4 | 25 |
| Platygastridae | 27 | 1.5 | 193 | 10.6 | 114 |
| Calliceratidae | 46 | 2.5 | 97 | 5.3 | 57 |
| Bethylidae | 175 | 9.6 | 0 | 0.0 | 0 |
| Embolemidae | 6 | 0.3 | 0 | 0.0 | 0 |
| Dryinidae | 12 | 0.7 | 5 | 0.3 | 3 |
| Cynipoidea | 5 | 0.3 | 13 | 0.7 | 8 |
| Mutillidae | 11 | 0.6 | 0 | 0.0 | 0 |
| Chrysididae | 3 | 0.2 | 0 | 0.0 | 0 |
| Totals | 1817 | | 1817 | | 1073 |

* These numbers have been increased, each in proportion, to compare directly the two populations on the basis of equal size, *i.e.*, the figures in this column are each increased by 69.5 per cent. from those in the following column as accurately as whole numbers will follow.

lection although it was represented to the extent of 9.6 per cent. by a series of highly specialized types in the amber. At the present time this family is unquestionably more abundant in warmer regions, which might account to some extent for a lesser abundance in the tanglefoot, although certainly not for its total absence, since it occurs very generally though sparingly in our region. As

this family is one which appears to represent a degenerate type related to the primitive wasps, this diminution in numbers is to be expected. One family, the Megalyridae, represented by a rather common extinct genus in the amber, occurs now only in Australia and South Africa and illustrates a condition noted among other groups of insects that certain types now surviving only in Australia or Malaya existed in the amber forests. Without going into further detail it is clear that the census of these parasitic Hymenoptera shows a quite consistently increased numerical abundance of phyloneanic types with a concurrent decrease in phylogerontic ones in the tanglefoot collection. Before attempting to evaluate these findings, I should like to call attention to another difference in the composition of the two faunas. If we examine the census figures with reference to the variety of types that make up the main bulk of the population[3] in the two faunas (Table III), we find that about one half the population of the tanglefoot belongs to a single family, the Ichneumonidae; two families make up almost two thirds; three make up three quarters and four make up seven eighths. In the amber population the number of families making up these several parts are 3, 4, 5 and 7. Thus more different types are well represented in the amber sample. Also, if we count all the families of parasitic Hymenoptera represented, we find

TABLE III
PARASITIC HYMENOPTERA

| | Amber | Recent |
|---|---|---|
| 50 per cent. of population | 3 families | 1 family |
| 66 per cent. of population | 4 families | 2 families |
| 75 per cent. of population | 5 families | 3 families |
| 87 per cent. of population | 7 families | 4 families |
| Entire population | 20 families | 12 families |

[3] In this way we may avoid a possible cause of error due to the certain rare types which may easily be absent in one sample and present in another.

twenty families in the amber (9 of these absent in tanglefoot) and only twelve in the tanglefoot (1 of these absent in the amber). The last data are not so convincing as the first, since the tanglefoot collection was smaller and several families might have been added if it had been as extensive as the amber one.

I can see only one conclusion to be drawn from these facts relating to the parasitic Hymenoptera. This group was more diversified in the amber forests and several primitive types were represented by more numerous individuals, while several derived ones were numerically less abundant than now. From the restriction in the number of larger groups we must conclude that this series was in a more active state of evolution in the upper Eocene than it is now, that certain gerontic types are being eliminated and that the population includes fewer dominant types. In other words, this group of insects is decadent from an evolutionary standpoint. We can not attempt to say whether the behavior of these insects has become more diverse, more highly adaptive or more complex, as we have only morphological data. However, the persistence of many amber genera to the present day combined with the considerable number of previously unknown structural types (including a new family) found in the amber make it seem very probable that a knowledge of the habits of these insects in the upper Eocene would show that their behavior was certainly as varied and complex as it is at the present day.

Among the Hymenoptera the ants furnish an extreme case of divergence between the amber and tanglefoot. The amber ants have been very carefully studied by Wheeler (1914), who examined 7,819 specimens from the collections of the University of Königsberg. This same collection yielded the 1817 specimens of parasitic Hymenoptera already mentioned. On this basis we should expect to find 467 specimens of ants in the tanglefoot collection if the ants had retained the same numerical ratio to the parasitic families. Instead, there are

only 52 ants, or about 11 per cent. of the expected number, indicating a great decrease in the ant population since the time of the amber, at least in this region. As ants certainly appear to be much more numerous in many tropical countries it appears that the numerical decadence of ants has not extended to the tropics. Professor Wheeler tells me that he believes a similar tanglefoot census in Australia, where ants are particularly numerous, might reveal a population as large as that of the amber forests. This in itself may be regarded as supporting the conclusion that ants are becoming less numerous since the Australian biota is on the whole the most archaic one now in existence.

If we tabulate the Coleoptera as we did the parasitic Hymenoptera, from the standpoint of population groups we find that there was a greater variety of types in the amber fauna (Table IV). Three families made up one

TABLE IV
COLEOPTERA

| | Amber | Recent |
|---|---|---|
| 50 per cent. of population | 3 families | 4 families |
| 66 per cent. of population | 9 families | 6 families |
| 75 per cent. of population | 12 families | 8 families |
| 87 per cent. of population | 22 families | 12 families |
| Entire population | 62 families | 39 families |

half of the population; nine made up two thirds; twelve made up three fourths and twenty-two made up seven eighths. For the tanglefoot the corresponding numbers are 4, 6, 8, 12. The amber data are for a larger collection than the tanglefoot, but it will be noticed that our table shows 62 families in the amber and only 39 in the tanglefoot. We have good reason to believe that diversification occurred much earlier among Coleoptera than among the other orders of holometabolous insects, at least with reference to the forest fauna[4] which occupies a very stable environment. If we are here concerned with an older

[4] *Cf.* Brues, '27.

group we must suppose that it is now further past its prime than the Hymenoptera, which I believe to be the case, especially as it formed only 2.5 per cent. of the whole insect population of the tanglefoot compared with about 4 per cent. in the amber.

It may now be asked how far we can extend these considerations to other groups of insects. This question may be approached first by a comparison of the numerical abundance of members of several other orders in the amber and tanglefoot. Unfortunately, I am unable to deal with the matter completely, as there is no reliable census of the amber fauna for several important groups. Those which may be considered are included in Table V.

TABLE V

| | Amber | Amber | Mean | Recent | Trend |
|---|---|---|---|---|---|
| | Per cent. | Per cent. | Per cent. | Per cent. | |
| Thyranura | 0.1 | 0.1 | 0.1 | 0.0 | − |
| Collembola | 6.4 | 10.6 | 8.5 | 0.2 | − |
| Trichoptera | 4.6 | 5.6 | 5.1 | 1.5 | − |
| Lepidoptera | 0.1 | 0.1 | 0.1 | 0.7 | + |
| Hemiptera | 3.1 | 7.1 | 5.1 | 10.8 | + |
| Diptera | 56.9 | 50.9 | 53.9 | 71.9 | + |
| Coleoptera | 3.6 | 4.5 | 4.0 | 2.5 | − |
| Hymenoptera | 3.4 | 5.1 | 4.2 | 5.0 | ± |
| Araneida | 3.7 | 4.5 | 4.1 | 4.5 | ± |
| Acarina | 6.7 | 8.6 | 7.6 | 0.05 | − |

The representation of these orders in the amber is given in percentages of the whole, based on two sources, and the average is in the third column. As these data do not distinguish between the several neuropteroid and orthopteroid orders, a number have necessarily been omitted. The data for the tanglefoot collection are in the fourth column and finally at the right has been added the general trend as to either increase or decrease in abundance. Four large groups, Thysanura, Collembola, Trichoptera and Coleoptera are clearly less numerous in individuals and three others, Lepidoptera, Hemiptera and Diptera are conspicuously more abundant. The Collem-

bola are a very primitive type. So are the Hemiptera, but it will be noticed in the tanglefoot collections that the great abundance of this order is due to a very large number of a single type, the leaf-hoppers. Contrasting Trichoptera with Lepidoptera, the latter are the more primitive and have decreased. Diptera show an enormous increase in spite of their great abundance already in the amber fauna. Clearly the Diptera are developing rapidly and we must look forward to them as the type that is well on its way to dominate the insect world. These statements refer entirely, of course, to population or abundance of individuals and although speciation or the number of specific types in the several groups frequently shows parallel deviations, such a correlation does not necessarily exist.

As to the proportionate abundance of the families of Diptera in amber we have no numerical statistics but many years ago the most astute Dipterist of his time, Hermann Loew (1861) examined a large series of amber Diptera. He states that among the Nematocera the Mycetophilidae (*s. lat.*) are the most numerous both in species and in individuals, while the Culicidae are the most poorly represented. This agrees with the tanglefoot series so far as it extends. Among Brachycera, Loew found that the Dolichopodidae far exceed all other families both in species and individuals. Next to this come the Empididae as far as species, but the number of individuals is far less. Here the tanglefoot figures are quite different. Although numerous, Dolichopodidae (3,070 specimens) are second to the Phoridae (6,443) which exceed any other family and form nearly one third of the whole tanglefoot collection of insects.

Phoridae are well represented in the amber, but are far less numerous than they are in the tanglefoot collection. The great abundance of this family is associated with the most diverse assortment of habits and behavioristic modifications, and of secondary morphological adaptations to be found in any group of Diptera. Yet these flies form a compact group with very distinctive charac-

ters of a highly modified and constant type. This suggests that the family, although well fixed as a group, is now in a very active stage of evolution. This conclusion is borne out by their very great present abundance compared with the amber.

Still another series of Diptera are exceedingly interesting in this connection. I refer to the larger muscoid flies, Muscoidea Thecostomata or Calyptratae, acclaimed by all workers as the most recent, most difficult and unstable of all Diptera. These Diptera are surpassed numerically by the smaller Muscoid flies, Muscoidea, Haplostomata or Acalyptratae, evidently a derivative of the other type. They exhibit enormous diversity in structure and appear to represent the group of Diptera destined to become most numerous in the future. Concerning the changes in abundance of these two groups since the amber we have no exact numerical data, but Loew states that the calyptrates were present although rare and that the acalyptrates were very poorly represented by only a small part of the recognized families. Contrasting this with the tanglefoot collection we find a great difference; 287 calyptrates and 1195 acalyptrates, comprising together 9 per cent. of all the Diptera obtained.

Numerous other groups of insects might be considered. True Neuroptera seem to have become less numerous, although not abundant in either fauna. Thysanoptera have clearly decreased; nearly 70 specimens have been studied by Priesner from the Königsberg and Fritsch collections, while only six appear in the tanglefoot, which is proportionately about half as many in the total population.

From the data presented we may conclude that within the rather narrow limits we have chosen, the insect population has manifested considerable change during its long transformation from the depths of the early tertiary amber forest to a modern one which has so far escaped destruction by the axe. There are many possibilities for error to creep into our methods of collecting the mate-

rials and of evaluating the resulting evidence, but the direction of change is clearly consistent among a number of different groups. We find that in the whole population certain components have increased, others have decreased and others have remained of about equal size. Parallel to the differentiation of genera and species there has been a correlated population increase in the more modern types of insects while the reverse has been true in groups that we may regard as primitive or decadent. The demonstration of an actual numerical increase in individuals in some groups and a decrease in others is the important fact which we may surmise but can not examine statistically in the case of other fossil insect faunas.

After this comparison of an Eocene and recent forest insect fauna can we still be sure that this is the age of insects? Are insects still on the increase in numbers and variety, or have they passed the heyday of their existence during tertiary times? We can not compare their numbers as a whole as we have no reliable measure of population density. We have seen, however, that certain groups have changed greatly in their ratio to the entire population, sometimes changing places with closely similar groups. Quite generally we find more specialized types replacing ones from which they seem undoubtedly to have been derived, although both appear commonly in each fauna. Meanwhile a few species here and there appear to have persisted throughout the entire period during which numerous genera and some families have disappeared completely, indicating a great fixity that promises little adaptive change in the future. In some groups that we have been able to compare in detail we find a greater diversity in the Eocene fauna than in the present one.

All these facts lead us to believe that many abundant groups of insects have passed their prime. We may still be in the age of insects, but certainly we do not now witness them coming into bloom.

In closing our discussion we may ask whether this slow process of changing insect populations has any bearing

on the present problems of applied entomology. So far as any influence on the changes which have occurred during the last century, we must undoubtedly answer in the negative. The upsetting of faunal balance through the establishment and spread of insect pests in new regions is a change of such magnitude and rapidity that it completely swamps the effect of any natural adjustments. Here without question the primary stimulus is extrinsic, involving a greatly increased food supply and a great diminution in natural checks to overpopulation. The changes we have considered appear to be wholly intrinsic, dependent upon the organisms themselves. Although they relate only to numerical abundance, they are analogous to and probably correlated with, if not dependent upon, the progressive morphological change characteristic of groups of animals whose descent can be traced accurately over long periods.

At the present time the disintegration of natural faunal areas has so far progressed that changes in insect populations can not be expected to follow any predictable path in the future.

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# PREDICTIONS AS TO CHROMOSOME CONFIGURATION, AS EVIDENCE FOR SEGMENTAL INTERCHANGE IN OENOTHERA[1]

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INASMUCH as Oenothera still remains the outstanding example of chromosome cohesion, it is of particular interest to the student of Oenothera to determine the causes behind this phenomenon. Some are fully convinced that segmental interchange is at the basis of chromosome cohesion, but others are skeptical. This paper is an attempt to present additional facts indicating the correctness of the segmental interchange theory as applied to Oenothera.

Translocations of a non-reciprocal type have been known in Drosophila since the work of Bridges in 1919. The idea of reciprocal translocations, or segmental interchange, was brought forward by Belling (1927) to account for the formation of a circle of 4 chromosomes in a hybrid between a standard line of Datura and a so-called cryptic type. At that time, Belling suggested that segmental interchange might be responsible for circle formation in Oenothera as well as Datura. This suggestion was followed up by Håkansson (1928) and particularly by Darlington (1929), who applied the theory to Oenothera in considerable detail.

A method of testing the segmental interchange theory was devised independently by Emerson and Sturtevant (1931) and by Cleland and Blakeslee (1930, 1931), and preliminary tests have already been published. It is the

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purpose of this paper to report further and rather extensive tests of the theory.

Before reporting upon the results of these tests, however, a word may be in order regarding the segmental interchange theory as it has been applied to Oenothera, and the method by which it may be tested. The theory assumes in the first place that the chromosomes of each so-called Renner complex have a definite and characteristic arrangement of end-segments, which may differ from those of other complexes. The two complexes composing a single form may, therefore, have different arrangements. The old idea that each chromosome of a given genom has a corresponding chromosome in the opposing genom is assumed to apply only in the case of paired chromosomes in Oenothera, and even here it is possible that homologies may be confined only to the ends. In the case of chromosomes found within circles, the assumption is that none of these chromosomes are strictly homologous with any other chromosome in the plant, but each chromosome is homologous, with respect to its end segments, to the 2 other chromosomes to which it is united in the circle (see Emerson 1931, Darlington 1929).

In the second place, it is assumed that these differences between complexes, as regards arrangement of homologous parts, have come about as the result of a series of segmental interchanges between non-homologous chromosomes, which have occurred at rare intervals during the evolutionary development of these complexes from a common source. As a matter of fact, segmental interchange is the only conceivable cause for differences in distribution of homologous parts, unless the position be taken that the various complexes now in existence, with their different arrangements, have had separate origins, and are devoid of evolutionary relationship.

The test devised by Emerson and Sturtevant and by Cleland and Blakeslee is really a method of proving that different arrangements of ends actually do exist in the various complexes. Since, however, such differences in

arrangement can only be explained on the basis of segmental interchange, the test may be taken as applying with equal force to the process of segmental interchange itself.

The method by which this test is applied is as follows: (1) A race with only paired chromosomes is selected as a standard line (*hookeri* deV. has been chosen by both sets of authors as the standard). (2) Various complexes are combined with the standard and with each other. From the chromosome configurations obtained in the resultant $F_1$ hybrids, it is possible to work out little by little the end arrangement in each complex. (3) In certain cases, this process can be aided by recourse to genetical data. (4) As each new complex is brought into the picture, it becomes increasingly possible to *predict* what configurations it will give in combination with certain complexes, as its behavior becomes known with certain others. (5) Such predictions will be fulfilled only provided the complexes involved have constant arrangements of ends, different complexes usually differing in respect to their arrangements. If these predictions are fulfilled, it proves that the assumption upon which they have been made is correct—namely, that end segments are differently distributed in different complexes and each complex has its own specific arrangement—facts explicable only on the basis of segmental interchange.

To date, 9 such predictions have been tested (Cleland + Blakeslee, '30, 1 pred.; Emerson + Sturtevant, '31, 2 preds.; Cleland, '32, 6 preds.). Every one of these predictions has been fulfilled, *i.e.*, each complex-combination has been shown to have the configuration predicted for it. No failures have been recorded. Such a record would seem to furnish strong evidence for the presence of different segmental arrangements in different complexes, and hence for segmental interchange.

During the past winter I have made a large number of additional predictions and verifications, and I propose to review these briefly at this time. A word may be

necessary, however, as to the mode of presentation. In order that the reader may see how the predictions were made, and how verified, it will be necessary to consider the data in the same order in which they were accumulated. There is admittedly no logic in the order in which data were obtained. Material was examined microscopically in the order in which it came to hand, plants blooming early in the season yielding on the whole the first fixations, the first embeddings and the first microscopic mounts. But my excuse in following this order is to show how the predictions were made and tested. It will be noticed that tests were made in every case subsequent to the making of predictions.

(1) The first hybrids examined were (*grandiflora* deV. x *franciscana* deV.) *acuta* and *truncata*. It had been predicted by Cleland (1932) that the *acuta* would have 2 ⊙s 4, 3 pairs, and the *truncata* ⊙ 14. These configurations were found to be actually present.

(2) The next forms examined were hybrids involving 3 California forms, known tentatively as $T + G$, *Devil's Gate* (Dev. G.) and *Dalton* (Dalt.). These quite distinct races will be described elsewhere. They each possess entirely paired chromosomes, so that all normal germ cells produced by them, either egg or sperm, will carry identical chromosome complexes from the standpoint of segmental arrangement. The first hybrids of these to be examined were (*grandiflora* $\times T + G$)*acuta* and *truncata*, and (*hookeri* $\times T + G$). The first had ⊙ 4 and 5 pairs, the second ⊙ 14, the third ⊙ 4 and 5 pairs. The next hybrid to be examined was *Dev. G.* $\times T + G$. It proved to have 7 pairs. This means that the complexes $^{h}$*Dev. G.* and $^{h}T + G$ have the same arrangement of ends, and consequently that hybrids of *Dev. G.* will have the same chromosome configurations as corresponding hybrids of $T + G$. Consequently it was possible to *predict* that $^{h}$*Dev. G. acuens* would have ⊙ 4 and 5 pairs, $^{h}$*Dev. G. truncans* ⊙ 14 and $^{h}$*Dev. G.* $^{h}$*hook.* ⊙ 4 and 5 pairs. All

3 of these were tested later in the season, and were found to have the predicted configurations.

The next hybrid studied was *Dalt.* $\times T + G$. This also possessed 7 pairs. *ʰDalt.* therefore has the same arrangement of ends as *ʰT* + *G* and *ʰDev. G.* One could therefore *predict* that *ʰDalt. acuens* would have ⊙ 4 and 5 pairs, *ʰDalt.truncans* ⊙ 14, *ʰDalt.ʰhookeri* ⊙ 4 and 5 pairs, and *ʰDalt.ʰDev.* G. 7 pairs. All 4 were later tested and found to have the required configurations.

(3) *Suaveolens* $\times T + G$ was then examined. Two complex-combinations were present, as expected, namely, *flavens.* *ʰT* + *G* and *albicans.* *ʰT* + *G*.

*Flavens.ʰT* + *G* proved to have 2 ⊙s 4 and 3 pairs. From this fact, it was evident that *flavens.ʰDev. G.* and *flavens.ʰDalt.* and their reciprocals must have 2 ⊙s 4 and 5 pairs. This was later found to be the configuration of these combinations.

*Albicans.ʰT* + *G* was found to have ⊙ 12 and 1 pair, from which it was predicted that *albicans.ʰDev. G.* and *albicans.ʰDalt.* would also show ⊙ 12 and 1 pair, which they did.

(4) Next in order of investigation after *suaveolens* $\times$ $T + G$ was (*chicagoensis* $\times$ $T + G$)*excellens.ʰT* + *G*. This hybrid turned out to have 7 pairs. From this it was possible to make 14 definitive predictions, as follows: *excellens.ʰDev. G.* and *excellens.ʰDalt.* should have 7 pairs, which proved to be the case. Furthermore, since *ʰT* + *G*, *ʰDev. G.* and *ʰDalt.* have the same arrangement of segments as *excellens,* they should give hybrids whose configurations are identical with those found in corresponding hybrids of *excellens. Excellens* is known in combination with *gaudens, punctulans, rubens, velans, flavens* and *ʰhookeri.* At the time that *excellens.* *ʰT* + *G* was found to have 7 pairs, the configurations of hybrids of *ʰT* + *G*, *ʰDev. G.* and *ʰDalt.* with *gaudens, punctulans, rubens* and *velans* were unknown. Since that time, however, 7 of the 12 hybrids whose configurations were thus

predicted have been tested. In every case, they have given the predicted configuration. These are:

| | |
|---|---|
| gaudens.$^{h}$T + G | ⊙ 10 |
| velans " | ⊙ 6 |
| gaudens.$^{h}$Dev. G. | ⊙ 10 |
| velans " | ⊙ 6 |
| gaudens.$^{h}$Dalt. | ⊙ 10 |
| velans " | ⊙ 6 |
| punctulans.$^{h}$Dalt. (metacline) | ⊙ 12 |

Of a total of 14 predictions, therefore, made from the fact that *excellens* gives 7 pairs with $^{h}T + G$, 9 have been tested, and all have shown the expected configuration.

(5) The next hybrid that was examined was *muricata* $\times T + G$. Two complex-combinations were found, namely *curvans.* $^{h}T + G$ (metacline) and *rigens.*$^{h}T + G$.

*Curvans.* $^{h}T + G$ was found to have ⊙ 4, ⊙ 8 and 1 pair. It was possible from this fact to *predict* that *excellens. curvans,* $^{h}$*Dev. G. curvans* and $^{h}$*Dalt. curvans* would also have a ⊙ 4, ⊙ 8 and 1 pair. Of these three, only one has been tested, namely, *curvans.* $^{h}$*Dalt.* This was found to have the required configuration.

*Rigens.*$^{h}T + G$ was predicted to have a circle of 8 and 3 pairs, on the basis of data available before the form was studied microscopically. The reasoning upon which this prediction was made is as follows:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Let $^{h}$hookeri = | 1·2 | 3·4 | 5·6 | 7·8 | 9·10 | 11·12 | 13·14 |
| " flavens = | 1·4 | 3·2 | 5·6 | 7·8 | 9·10 | 11·12 | 13·14 |
| " velans = | 1·2 | 3·4 | 5·8 | 7·6 | 9·10 | 11·12 | 13·14 |

$^{h}T \times G$ gives ⊙ 4 with $^{h}$*hookeri,* 2 ⊙s 4 with *flavens* and ⊙ 6 with *velans.* It therefore has in common with $^{h}$*hookeri* 2 chromosomes which are not present in *flavens.* The only two $^{h}$*hookeri* chromosomes which are not found in *flavens* are 1·2 3·4. $^{h}T + G$ must have these, therefore, and having them, will give ⊙ 4 as part of its configuration with *flavens.* In order to give a ⊙ 4 with $^{h}$*hookeri,* an additional ⊙ 4 with *flavens* and ⊙ 6 with *velans,* $^{h}T + G$ must differ from $^{h}$*hookeri* and *flavens* by a single interchange between one, but not both of the

chromosomes which are involved in the circle in *hookeri. velans,* and one of the last three chromosomes. Defining 7 · 8 and 9 · 10 as the *hookeri* chromosomes involved in the circle with $^{h}T + G$,[2]

Let $^{h}T+G=1\cdot2$ $3\cdot4$ $5\cdot6$ $7\cdot10$ $9\cdot8$ $11\cdot12$ $13\cdot14$

*Rigens* gives ⊙ 6 with *hookeri,* and ⊙ 4, ⊙ 6 with *flavens.* It therefore has 2 more chromosomes in common with *hookeri* than with *flavens.* These must be the 2 *hookeri* chromosomes which *flavens* does not have. *Rigens* must therefore have 1 · 2 3 · 4. Again, *rigens* gives ⊙ 8 with *velans,* and ⊙ 6 with *hookeri.* It therefore has one more chromosome in common with *hookeri* than it has with *velans.* The only chromosomes which *hookeri* has which are not common to *velans* are 5 · 6 and 7 · 8. *Rigens* has therefore either 5 · 6 or 7 · 8 (not both).

(a) If *rigens* has 5 · 6, it can not have 7 · 8, so can not have both of the *hookeri* chromosomes which are involved in the *hookeri* · $^{h}T + G$ circle. It can, however, have (1) one, or (2) neither of these chromosomes, as in the following hypothetical formulae.[3]

rigens (1) 1 · 2 3 · 4 5 · 6 7 · 12 9 · 10 11 · 14 13 · 8 (would give ⊙ 8 with $^{h}T + G$)

rigens (2) 1 · 2 3 · 4 5 · 6 7 · 10 9 · 12 11 · 8 13 · 14 (would give ⊙ 4 with $^{h}T + G$)

(b) If *rigens* has 7 · 8, it already has one of the chromosomes in the *hookeri* · $^{h}T + G$ circle, and can have the other or not. If it has both in common with *hookeri,* its formula may be arranged as follows:[3]

rigens (3) 1 · 2 3 · 4 5 · 12 7 · 8 9 · 10 11 · 14 13 · 6 (would give ⊙ 4, ⊙ 6 with $^{h}T + G$)

If it has but one of these two chromosomes in common with *hookeri* (7 · 8), the formula may be written:[3]

[2] The subsequent reasoning will apply no matter what choice is made within the limits stated. In this, as in the later choices made in the present line of reasoning, no claim is made that the choice in each case will turn out to be the correct one.

[3] Subsequent reasoning will apply, no matter how the ends of the *rigens* chromosomes which enter into the circle of 6 in *rigens* · *hookeri* are chosen.

rigens (4) 1·2 3·4 5·10 7·8 9·12 11·6 13·14 (would give ⊙ 8 with ʰT + G)

There are then 3 tentative possibilities for *rigens*· ʰ*T* + *G*, namely, ⊙ 4; ⊙ 4, ⊙ 6; and ⊙ 8. These possibilities may be tested in part by the use of *acuens*. *Acuens* gives ⊙ 4 with ʰ*T* + *G* and ⊙ 4, ⊙ 6 with *velans*. It therefore has only 3 chromosomes in common with ʰ*T* + *G* which are not present in *velans*. There are only 3 ʰ*T* + *G* chromosomes, however, which are not found in *velans*, namely 5·6 7·10 9·8. *Acuens* must therefore have these. But *acuens* gives ⊙ 4, ⊙ 8 with *rigens*, having but one chromosome in common with the latter. This at once eliminates *rigens* formula (2), since this has 2 of the 3 chromosomes which *acuens* must have. *Rigens*.ʰ*T* + *G* can not have, therefore, ⊙ 4 and 5 pairs. Hypothetical *acuens* formulae can be written, however, which will fit the other *rigens* formulae, as well as all other formulae known, as follows:

acuens = 1·4 3·2 5·6 7·10 9·8 11·12 13·14 (This will fit rigens (1) and (4))

" = 1·2 3·14 5·6 7·10 9·8 11·12 13·4 (This will fit rigens (3))

By the use of *stringens*[4] it is possible to eliminate one of the tentative *acuens* and hence one of the remaining possibilities for *rigens*. *Stringens* has 1·4 and 3·2 (Cleland and Blakeslee 1931), and gives 2 ⊙s 4 with *acuens*. It is possible to construct a *stringens* which will give the correct configuration with ʰ*hookeri*, *flavens* and *velans*, and also with the first formula of *acuens*, as follows:

stringens = 1·4 3·2 5·12 7·8 9·10 11·6 13·14

It is impossible, however, to formulate a *stringens* which will give only circles of 4 with the second formula for *acuens*, for the chromosomes 1·4 and 3·2 of *stringens*

[4] At the time this prediction was made, it was not known that *acuens*·*flavens* has ⊙ 4 and 5 pairs. With that information available, the second *acuens* formula would have been automatically eliminated, as giving the wrong configuration with *flavens;* and *stringens* would not have been needed in the argument.

would perforce belong to a circle of larger size if brought into combination with a complex in which one of the first two chromosomes has exchanged with a chromosome further along in the formula, as in the second formula for *acuens*. Consequently, the second formula for *acuens* is impossible. This means, however, that the third formula for *rigens* is also impossible. Only 2 formulae remain for *rigens*, therefore, and both give a circle of 8 with *h*$T + G$. It was possible, on the basis of this line of reasoning, therefore, to *predict* that *rigens.h*$T + G$ would have a circle of 8. This would also mean that *rigens.h Dev. G.* and *rigens.h Dalt.* should have a circle of 8. All 3 forms were tested microscopically, and were found to have the required configurations.

(6) The last hybrids to be examined were *Dev.* $G \times$ *franciscana* deV. and $T + G \times$ *franciscana* deV. Since *h franciscana* deV. has the same end arrangement as *h hookeri*, it follows that it must give the same configuration with *h Dev. G.* and *h*$T + G$ that *h hookeri* gives, namely, ⊙ 4 and 5 pairs. Upon examination, these hybrids were found to have this chromosome arrangement.

This completes the list of predictions made and tested during the past winter. They make a total of 28 predictions, every one of which was found to be correct. These added to the 9 predictions previously published make a grand total of 37 predictions which have been tested, every one of which has been proved correct.[5]

Of what significance, then, is this accomplishment? It seems to me that it takes segmental interchange definitely out of the realm of theory and places it in the realm of proved fact. For in the first place, these results indicate that each complex has its own specific and characteristic arrangement of end segments, that different complexes may have different segmental arrangements, and that the

[5] It should be possible, more and more, as individual genes are assigned to particular segments, to predict genetical linkages in given complex-combinations, as well as chromosome configurations. A beginning along this line has been made by Emerson and Sturtevant (1931).

union of homologous ends will give a definite, characteristic and predictable arrangement of chromosomes in each particular complex-combination. Second, the only possible way to account for the different arrangements of ends in different complexes is, as we have seen, on the basis of mutual exchanges between non-homologous segments—in other words, segmental interchange. In the third place, definite predictions such as these are impossible on any other grounds than those here assumed. It would seem therefore that the data at hand furnish a definite proof of the validity of the segmental interchange theory as applied to Oenothera.

I wish to call attention, in conclusion, to the effect of this work upon the problem of genetical linkage in Oenothera. The field has been contested by 2 opposing theories. The first, the so-called chromosome cohesion theory (Cleland 1923, 1924, 1926), has assumed that each individual univalent chromosome occupies a definite position within the circle, with paternally and maternally derived chomosomes alternating; that paternal and maternal chromosomes are separated as a rule to opposite poles, inasmuch as adjacent chromosomes are known to go to opposite poles; that the uniform passage of all paternal chromosomes to one pole and maternal chromosomes to the other pole can mean but one thing genetically—a linkage between genes in non-homologous chromosomes belonging to the circle. The extensive linkage found in Oenothera and the formation of the Renner complexes are therefore ascribed to chromosome linkage. The alternative theory denies the definite position of individual chromosomes (Shull 1928), claiming that, while homologous chromosomes are probably adjacent in the circle, they may occupy either the right-hand or the left-hand position indifferently, and consequently independent assortment will take place between different sets of homologues as readily when these are within a single circle as when they belong to different chromosome groups. Chromosome linkage has therefore nothing to

do with genetical linkage, all of which is to be explained in the ordinary and conventional way—as due to inclusion within a single chromosome.

But if the segmental interchange theory be correct, then each particular chromosome *must* occupy a given and specific place with reference to the other chromosomes in the circle, for it *must lie adjacent to those chromosomes which have ends homologous with its own.* If segmental interchange be proved correct, then determinate chromosome position is a proved fact, and the only element of doubt that has been present in the argument for the chromosome cohesion theory is thereby removed. If then the evidence from predictions, as I have outlined it, is accepted as a sufficient proof of segmental interchange in Oenothera, it follows automatically that chromosome cohesion is responsible for the linkage of genes in non-homologous chromosomes of a circle. Even if the present verdict in regard to segmental interchange is "not as yet proven," one must at least admit that the concept of segmental interchange inevitably implies that each chromosome of a circle occupies a determinate position within the circle; and if and when segmental interchange becomes accepted as a fact, it will be necessary to accept along with it the chromosome cohesion theory.

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# THE RELATION OF THE INDUCED MUTATION RATE TO DIFFERENT PHYSIOLOGICAL STATES IN DROSOPHILA MELANOGASTER: II. IRRADIATION DURING COMPLETE ANESTHESIA[1]

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IN investigation of the manner in which radiation effects are produced, the authors undertook to study the possible influence of various internal factors acting along with radiation. The present paper constitutes the second of a series dealing with radio-physiology.

As has been pointed out in the first paper of this series (in press), and others, there can be little doubt that tissues and cells differ in susceptibility and that the sensitivity of a given cell may change from time to time. The same intensity of radiation produces very dissimilar effects upon different kinds of cells and upon cells in different phases of activity or of their life histories. In 1906, Bergonié and Tribondeau (1)[2] formulated the principle, known as the first law of radiation therapy, that susceptibility varies directly with reproductive capacity and indirectly with the degree of differentiation. Observations by many investigators indicate the high sensitivity of rapidly growing tissues and of cells undergoing the complicated process of division. (2, 6, 8, 9, 10, 11).

Canti and Donaldson (2), studying the effects of radiation on mitosis *in vitro,* found that even a brief exposure to the gamma-rays of radium prevented cells which were about to divide from beginning the process. Unless, however, irradiation is prolonged or very intense, most cells suffer no visible injury but begin to divide again after a short recovery period, giving no evidence of per-

[1] The expenses of this investigation were supported in part by a grant from the committee on the effects of radiation upon living organisms of the National Research Council.

[2] Items of the literature cited in the first paper are referred to by number.

manent harm. This return to activity after relatively slight exposure, and the fact that in cells under irradiation some part will respond more quickly than another, form the basis of the entire field of those genetic investigations in which lethal and visible mutations, translocations and gene-rearrangement have been produced experimentally in an attempt to solve the problem of the causes of variability among organisms in nature.

In the early days of the therapeutic application of x-rays and radium, the term "selective action" was employed as representing that the rays produced a greater effect upon some cells than upon others. As irradiation gradually came into use both therapeutically and experimentally, observations pointed more and more to tissue and cell specificity, and it came to be generally accepted that this action is not due to any selective power of the rays but to the susceptibility of the cells. Early in radiation therapy the endothelium of the blood vessels was found to be one of the most sensitive of tissues. Finzi and Horsley (1911) in work on the brain came to the conclusion that the changes observed were due almost entirely to changes in the blood vessels, while the direct action of relatively large doses on the cerebral tissues seemed to be extremely slight. That glandular tissue is affected readily was shown as early as 1909 by Dominici's experiments on the skin. Finzi (1913) found gland tissues susceptible to even slight action of x-rays and radium, the cells disintegrating almost at once and gradually being removed by leucocytes. The high sensitivity of gonadial tissues was also recognized early.

Thus it appeared that the term "selective action" focused too much attention upon the radiation and too little upon the response of the thing irradiated, and the terms "sensitivity," "vulnerability," "susceptibility" were used as substitutes. These have been followed by the term "selective resistance," intending to indicate that cells would be more or less affected by radiation, according to their ability to oppose a large or small resistance to its action. The term "selective absorp-

tion" was suggested, but it is now generally accepted that the coefficient of absorption for all kinds of cells, determined by a progressive comparison with that of different metallic screens, is about the same.

Whether the cell is affected at all or what part of it is altered seems to depend upon the physiological condition at the time of irradiation and on the consequent physical and chemical changes. Very little is known as yet of the chemistry of irradiated material, but there is reason to believe that the cell constituents are ionized and cellular ferments activated, so that reactions are possible which otherwise would not occur at all. In different sections both of the same malignant growth and of normal tissues taken before and after irradiation, the staining reactions point to marked changes in chemical composition. Recent investigations (Love, 1931, Spear, 1932) of the effects of radiation *in vitro* indicate that a limit of action of x-rays and radium exists beyond which no further cells are affected as a result of exposure. Love in 1931 found that the diminution curve of dividing cells reaches a plateau and that in order to obtain further reduction relatively greater doses were necessary. It is conceivable, perhaps, that a limit of effectiveness is reached, because at that point all cells in such a physiological state as would render them susceptible have been affected.

### The Influence of Complete Anesthesia

In an attempt to study radiation effects under varying physiological conditions, both males and females of Drosophila were irradiated during complete anesthetization by di-ethyl ether. Exposure was effected in thin gelatin capsules, enclosed in a small double-walled chamber, the bottom and sides of which were constructed of very thin wood. The chamber was open at the top. Between the walls cotton was kept saturated with ether in sufficient concentration that the flies were "just under" the anesthetic throughout treatment. As a reservoir for the ether, a modified Mariotte bottle was employed which was equipped with a U-tube and capillary

thermometer tubing, the flow of ether being restricted thus to a series of timed drops. The small anesthetization chamber containing the capsules was placed as usual in a larger irradiation chamber, the latter being entirely enclosed except for the passage of small-bore rubber tubing which served to carry the anesthetic fluid. Within the irradiation chamber thermometer tubing was not used in order to avoid secondary radiation from the glass.

### ETHERIZED IRRADIATED MALES, ETHERIZED IRRADIATED FEMALES, AND IRRADIATED NON-ETHERIZED CONTROLS

Here, as in experiments previously reported, the test of radiation effectiveness was the rate of induced lethal mutation occurring among the descendants of crosses of ClB (sc, v, f, bb) by wild. Wild-type males, newly hatched from cultures at the height of productivity, were exposed under anesthetic in this way to an irradiation of 300 milligram-hours (gamma-radiation only) at a distance of ten centimeters. The alpha and beta-rays were excluded by means of a 0.5 mm platinum filter, which at the same time allows the passage of 78 per cent. of the gamma-radiation. Supplementary filtration through a layer of Columbia paste one centimeter in thickness was used. Following exposure, these males were mated to ClB females. Lethal mutations induced in the treated parent generation are detected in the $F_2$ generation.

The treated females were first generation bar-eyed individuals of the cross, ClB (sc, v, f, bb) by wild. These females receive one x-chromosome carrying the ClB factor and one x-chromosome bearing the wild-type characters. The sc, v, f, bb-chromosome from the female parent goes to the first generation sons, which in this case are discarded. The first-generation females are heterozygous for the lethal and when mated to the wild-type give ordinarily one half the usual number of sons in the $F_2$ generation, or a 100:50 ratio. A new lethal mutation induced in the chromosome bearing the wild-type characteristics is inherited by the other half of the

sons, and they also fail to appear. As was indicated in the first paper of this series, a number of preliminary experiments demonstrated the occurrence of such an induced lethal in this x-chromosome in proportion to the dosage applied. Such carrier females were irradiated under anesthetic in exactly the same manner as the males. Here the lethal is detected in the generation immediately following treatment.

Both males and females were compared with controls irradiated under identical conditions of treatment—distance, filtration, dosage, exposure time, etc., being the same.

### Anesthetized Controls

Previous to Muller's discovery in 1927 that mutations could be produced experimentally by x-radiation, many different kinds of chemicals had been tried without success. Notwithstanding this fact, a series of control experiments was made in order to be certain whether long-continued anesthetization in the absence of irradiation could bring about lethal mutation. Wild-type males and females of ClB by wild were anesthetized separately for a period of one hour and mated afterward in the usual manner. In 2,450 $F_2$ cultures of etherized males and 2,500 corresponding cultures from etherized females no lethal mutations occurred.

### Experimental Findings

Unlike the results from flies irradiated after starvation, no post-irradiative mortality was observed here in the treated parent generation. The comparisons given in Table I and Fig. 1 show at once the high sterility and lethal mutation percentages occurring among the descendants of flies exposed to gamma-radiation under complete anesthesia. The differences in both series of irradiation, that of treated males and that of treated females, are well above the threshold of significance.

The sterility values are again absolute, representing complete sterility. As in experiments previously re-

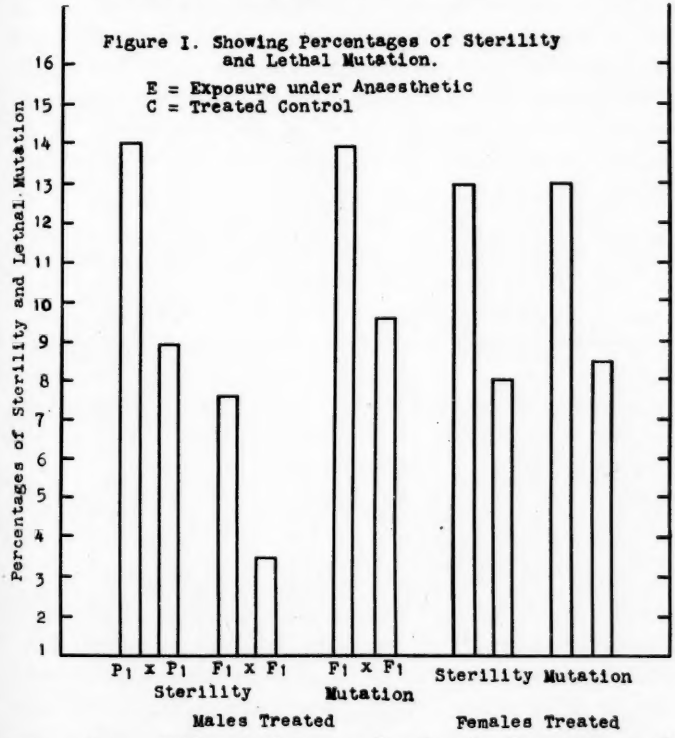
TABLE I

SHOWING THE PERCENTAGES OF STERILITY AND LETHAL MUTATION FOLLOWING IRRADIATION OF DROSOPHILA UNDER ANESTHETIC AS COMPARED WITH TREATED CONTROLS

| Generation | Exposure under anesthetic | No. cultures | Treated control | No. cultures | Difference | Significance |
|---|---|---|---|---|---|---|
| | | | Per cent. of Sterility | | | |
| Treated males | | | | | | |
| $P_1 \times P_1$ ..... | 14.0067 ± 0.6072 | 1485 | 8.9887 ± 1.4451 | 178 | + 5.0180 ± 1.5620 | 3.21 × p. e. |
| $F_1 \times F_1$ ..... | 7.5319 ± 0.3671 | 2350 | 3.6923 ± 0.4988 | 650 | + 3.8396 ± 0.6192 | 6.21 × p. e. |
| | | | Per cent. of Lethal Mutation | | | |
| $F_1 \times F_1$ ..... | 13.9910 ± 0.4954 | 2230 | 9.7444 ± 0.7980 | 626 | + 4.2466 ± 0.9392 | 4.52 × p. e. |
| | | | Per cent. of Sterility | | | |
| Treated females | 12.9333 ± 0.4833 | 2250 | 8.0000 ± 0.4724 | 1500 | + 4.9333 ± 0.6758 | 7.29 × p. e. |
| | | | Per cent. of Lethal Mutation | | | |
| | 13.0168 ± 0.5060 | 1959 | 8.4782 ± 0.5085 | 1380 | + 4.5386 ± 0.7174 | 6.33 × p. e. |

Total number of initial single-pair matings on which these figures are based: Series of Treated Males—3,835; Series of Treated Females—3,976. Sterility tests are additional to these figures.

ported, all matings giving indication of non-fertility were allowed a period of recovery at the end of which time sterility tests were made. It is interesting to note in the results for treated males, where the lethal is carried by the $F_1$ female and comes to light in the second generation, a confirmation of earlier findings, *i.e.*, that the $F_1$ sterility values are approximately one half those for the treated




generation. This consistent reduction in the amount of sterility occurring among individuals two generations removed from exposure suggests perhaps the existence of some factor necessary for fertility which was affected by the treatment. The writers hope to test this point in the near future.

Concerning the slightly different response of the two sexes to identical conditions of irradiation, Table II

reveals that the sterility and mutation values are consistently less when females are treated, although not significantly so. Indications of sex differences have been found by Muller (7, 1929) in a number of experiments with x-rays. The rate of induction in mature sperm was observed to be higher than that in adult females; larval males gave a higher rate of mutation than females rayed at the same larval stage. A greater sex difference in sensitivity was apparent in gene-rearranging effects of the rays than in transmuting effects on individual genes.

The consistent high percentages of sterility and the increased lethal mutation rates obtained in these experiments by irradiation of flies during anesthesia are un-

TABLE II

SHOWING SLIGHT DIFFERENCES IN THE RESULTS OBTAINED WHEN MALES AND FEMALES ARE IRRADIATED UNDER IDENTICAL CONDITIONS OF TREATMENT

| Percentage of | Males treated | Females treated | Difference |
|---|---|---|---|
| | Treated control | | |
| Sterility | 8.9887 ± 1.4451 | 8.0000 ± 0.4724 | 0.9887 ± 1.5133 |
| Mutation | 9.7444 ± 0.7980 | 8.4782 ± 0.5085 | 1.2662 ± 0.9462 |
| | Exposure under anesthetic | | |
| Sterility | 14.0067 ± 0.6072 | 12.9333 ± 0.4833 | 1.0734 ± 0.7760 |
| Mutation | 13.9910 ± 0.4954 | 13.0168 ± 0.5060 | 0.9742 ± 0.7081 |

doubtedly significant. It is difficult, however, to say to what extent the differences observed were due to increased sensitivity of the gonadial tissues under anesthetic and to what extent, if any, to mechanical effects. Early in the experimental work it was thought that some secondary radiation might result from the small etherization chamber or its rubber tube attachment. Consequently, controls were irradiated in the chamber with its attachment but in the absence of any anesthetic fluid. The resulting sterility and mutation percentages accord almost exactly with those of the original treated controls, thus eliminating that possibility. Whether increased secondary radiation from the high concentration

of ether vapor within the enclosed treatment chamber might be in part responsible is a question at the present stage of the experiments.

It is conceivable perhaps that ether absorbed by the germ cells may be a sensitizer in combination with radium-radiation and may have a catalytic action for the type of chemical transformation which results in the production of lethal mutation. Changes in protoplasmic viscosity may be brought about by irradiation which might have considerable influence upon the response of the cell to absorbed chemical substances. Haendly (1918), studying changes in irradiated carcinoma cells, observed marked alterations of the degree of hydration and of viscosity, increase or decrease in nuclear size, aggregation or disintegration of chromatin, etc. That sol $\rightleftarrows$ gel changes occur in the colloidal constituents of nerve cells during anesthesia is generally accepted. Possibly it is not running too far afield to suppose that similar changes in germ cells might sensitize them to radium-irradiation.

The above suggestions are offered merely as an attempt to explain the phenomena observed in our experiments. The results obtained pave the way for similar studies of a more detailed character in analysis of just how the effect is produced. Accordingly, the experiments are being continued with the idea of studying the physical agents employed and the induced physiological conditions of the animals.

## Summary

In preliminary studies of the physiology of mutation production, males and females of Drosophila were irradiated during complete anesthesia by di-ethyl ether. Unlike the results reported in the first paper of the series where the flies had been irradiated after starvation, both males and females exposed under anesthetic to gamma-radiation of 300 milligram-hours showed no mortality in the treated parent generation, but gave high percentages of sterility and a high rate of lethal mutation as compared with treated controls. In controls exposed to an-

esthetic alone no signs of sterility were observed, and no lethal mutations occurred. There was a slight difference in the response of the two sexes to identical conditions of irradiation, the females being consistently a little less sensitive. These differences are, however, not statistically significant. The experiments are being continued with a view to analyzing just how the effect is produced and to determining to what extent the results obtained are due to increased sensitivity of the gonadial tissues and to what extent, if to any, to mechanical effects.

The authors take pleasure in expressing their appreciation to Drs. A. Lacassagne and G. Gricouroff, of the Radium Institute, and to the Radiological Clinic of the Curie Foundation for arranging the radium exposures.

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# GENIC MODIFICATIONS IN DROSOPHILA MELANOGASTER INDUCED BY HEAT IRRADIATION

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MUTATIONS induced in 5–6 day old larvae of *Drosophila melanogaster* by exposure to a high temperature have been reported by Goldschmidt, Jollos and Rokizky. Ferry, Shapiro and Sidoroff, in their experiments, which were a repetition of Goldschmidt's work, failed to find any mutations. In order to offer additional information relative to the effect of high temperature on the mutability of *D. melanogaster,* the authors repeated Goldschmidt's experiments with slight modifications suggested by Jollos. Further information was sought relative to the response of variously aged larvae, as well as the adults themselves, when exposed to high temperature.

A Florida culture of wild type *D. melanogaster* was closely inbred for three generations before a stock was made up for the heat irradiation experiments. Seventy-two flies were paired, and three pairs were placed in each of 12 vials, measuring $1 \times 4$ inches, which had been quarter filled with banana agar. After 24 hours the flies were transferred to fresh vials, and this process was repeated until five sets of 12 vials each were obtained. Variously aged larvae, 1–2, 2–3, 3–4, 4–5 and 5–6 days old, respectively, were then available for experimentation.

Ten vials of each set were treated at 36° C. for 15 hours, the other two vials of each set being reserved for untreated controls. Immediately after the exposure the vials were emptied into half pint bottles, containing a

[1] The authors are grateful to Dr. D. E. Lancefield, of the department of zoology, Columbia University, for helpful suggestions, and to Dr. C. R. Plunkett, of New York University, for a culture of Florida wild type *Drosophila melanogaster.*

corn-meal-syrup-agar culture medium. The immediate addition of fresh food undoubtedly greatly reduced the mortality rate of the larvae, which otherwise would have had to live in over-fermented food. All flies were kept at 26° C. before and after the exposure to 36° C.

As soon as the flies hatched, mass cultures were made, usually placing three or four pairs of flies in a culture bottle, though occasionally as many as five pairs were bred in a single bottle. It is quite possible that the bottles were overcrowded and that some mutants, which are presumably weaker than the wild type, were lost. Very few bottles, however, failed to yield numerous flies, and comparatively few bottles were discarded on account of excess bacterial or mold growths.

Flies were obtained from 13 of the 50 cultures treated in five series of 10 cultures each. In the series in which the larvae were 1–2 days old when treated, four cultures yielded adults; 2–3 days old, one culture; 3–4 days old, none; 4–5 days old, two cultures; and 5–6 days old, six cultures. Disregarding the cultures which failed to yield any adults at all, the average number of flies per vial for 1–2 day old larvae was 7.3; 2–3 day old, 14; 3–4 day old, 0; 4–5 day old, 15.5; and 5–6 day old, 3.7, while the controls averaged 63.6 flies per culture. The mortality rate among the treated flies was relatively high. The total number of treated larvae which hatched into viable adults was 96. In the progeny of these flies a total of 8 distinct mutations were discovered, an additional mutant, sooty, showing in the hatched larvae. Tables I and II present data which show the number of cultures and flies included in the experiment, and the number of the genic modifications obtained, respectively.

The mutations arose in the following numbers: Among the 29 viable 1–2 day old larvae, 6; the 14 viable 2–3 day old larvae, 2; (no 3–4 day old larvae hatched); the 31 viable 4–5 day old larvae, 1; and the 22 viable 5–6 day old larvae, 0. Apparently the larvae which were 1–2 days old when exposed to heat irradiation responded to temperature change to a greater degree than the other

TABLE I

THE AGE OF LARVAE OF DROSOPHILA MELANOGASTER TREATED AT 36° C. FOR 15 HOURS, THE NUMBER THAT SURVIVED AND THE NUMBER OF THEIR OBSERVED PROGENY

| Age of larvae | Number cultures yielding flies | Number flies hatched | Number flies in $F_1$ | Number flies in $F_2$ | Number flies in $F_3$ |
|---|---|---|---|---|---|
| 1–2 day | 4 | 29 | 2,088 | 7,906 | 7,092 |
| | *2 | 118 | 219 | 1,444 | 1,037 |
| 2–3 days | 1 | 14 | 1,041 | 6,664 | 4,070 |
| | *2 | 117 | 2,621 | 2,597 | 1,119 |
| 3–4 days | 0 | ..... | ..... | ..... | ..... |
| | *2 | 104 | 1,420 | 1,424 | 2,526 |
| 4–5 days | 2 | 31 | 910 | 5,289 | 3,370 |
| | *2 | 442 | 628 | 2,070 | 1,314 |
| 5–6 days | 6 | 22 | 273 | 224 | 531 |
| | *2 | 130 | 267 | 233 | 631 |
| Totals | 13 | 96 | 4,312 | 20,083 | 15,063 |
| | *10 | 911 | 5,155 | 7,768 | 6,627 |

* Untreated control cultures.

groups of variously aged larvae which were exposed to the same treatment.

The same original stock of wild type *D. melanogaster* was used for testing the effects of high temperature on the mutation rate of the adults. Fifty flies were placed in individual glass test-tubes, which were tightly corked and submerged in a constant temperature water bath at the desired temperature for specific lengths of time. Though a total of 8,618 flies were treated, only 1,140 were tested for mutations.

Three additional mutations were found to have occurred among the offspring of these adult flies. The temperatures used and the length of each exposure together with the number of flies treated and the number of their progeny examined are presented in Table III. Of the nine series which were treated, three yielded mutations, sooty, sepia and abnormal legs, respectively.

TABLE II

GENIC MODIFICATIONS INDUCED IN LARVAE OF DROSOPHILA MELANOGASTER AT 36° C. FOR 15 HOURS

| Cultures | | Culture No. 1 | Culture No. 2 | Culture No. 3 | Culture No. 4 | Culture No. 5 | Culture No. 6 | Controls for cultures | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Larvae 1–2 days old | Larvae 1–2 days old | Larvae 1–2 days old | Larvae 2–3 days old | Larvae 4–5 days old | No. 1–4 | No. 5 | No. 6 |
| Modifications found in Florida wild stock | | Deformed eye<br>Bristle modifications | | | | | | | | |
| Induced modifications | | White $F_2$<br>Abnormal legs $F_3$ | White $F_2$<br>Abnormal legs $F_3$ | Sepia $F_2$<br>..... | Sepia $F_2$<br>..... | White $F_2$<br>Sooty $F_2$ | Sooty p<br>..... | ..... | ..... | ..... |
| Number of flies counted | $F_1$ | 1,366 | 262 | 152 | 308 | 1,041 | 248 | 219 | 2,622 | 628 |
| | $F_2$ | 4,140 | 1,575 | 924 | 1,267 | 6,664 | 1,958 | 1,444 | 2,597 | 2,070 |
| | $F_3$ | 1,709 | 2,393 | 1,408 | 1,582 | 4,070 | 1,755 | 1,332 | 1,119 | 1,314 |

TABLE III

THE TEMPERATURES, LENGTHS OF EXPOSURES, INDUCED MUTATIONS AND THE COUNTS OF THE PROGENY OF ADULT DROSOPHILA MELANOGASTER EXPOSED TO HEAT IRRADIATION

| Length of exposure | Temperature °C. | Number flies used | Number flies in $F_1$ | Number flies in $F_2$ | Number flies in $F_3$ | Induced mutations |
|---|---|---|---|---|---|---|
| 5 min. | 43 | 41 | 1,662 | 1,818 | 3,872 | 0 |
| 5 min. | 42 | 100 | 2,350 | 6,279 | 6,523 | 0 |
| 10 min. | 41 | 183 | 5,096 | 2,309 | 3,422 | 1, sooty, $F_2$ |
| 15 min. | 41 | 46 | 893 | 1,287 | 1,079 | 0 |
| 20 min. | 40 | 195 | 566 | 807 | 683 | 0 |
| 30 min. | 40 | 188 | 344 | 1,164 | 2,232 | 0 |
| 40 min. | 39 | 196 | 589 | 747 | 283 | 0 |
| 60 min. | 39 | 173 | 7 | 301 | 979 | 1, sepia, $F_3$ |
| 14 hrs. | 36 | 18 | 293 | 1,288 | 1,304 | 1, abnormal legs, $F_2$ |
| Controls | | 342 | 5,032 | 5,468 | 6,818 | 0 |

A total of twelve distinct mutations was obtained from the heat-treated flies observed in this test, which included a count of over 110,000 flies. Approximately 45,000 untreated flies were examined for mutations, but none were found. Many irregularities were noted, but they were found either to occur among the untreated flies or to be abnormalities that are not inherited. Among these flies eye, bristle, wing and abdomen deformities were found, many of which corresponded to the forms recorded by Goldschmidt, Jollos and Rokizky. In this connection Table IV presents comparative lists of modifications found by Goldschmidt, Jollos, Rokizky and the authors.

The four genic modifications herewith reported, namely, white, sooty, sepia and abnormal legs, were tested through $F_2$ and $F_3$ for inheritance and were found to be hereditable genic modifications. The white eye individuals which appeared in three independent cultures were all males. They were crossed to wild type and to

TABLE IV

COMPARATIVE LISTS OF REPORTED GENIC MODIFICATIONS IN DROSOPHILA MELANOGASTER INDUCED BY HEAT IRRADIATION

| Goldschmidt | Jollos | Rokizky | Authors |
|---|---|---|---|
| (Florida wild stock) | (Florida wild stock) | (Florida wild stock) | (Florida wild stock) |
| Aristapedia | Eosin | Rough eye | White |
| Sooty | Eosin modification factor | Dark body and darker trident pattern | Sooty |
| Kidney | Black or Sooty | | Sepia |
| Rolled | | Notch | Abnormal legs |
| White | Bobbed | Crinkled wings | |
| (And many others) | Yellow | Reduced bristle size | |
| | Abnormal abdomen | Lethal | |
| | (Spineless stock) | Rudimentary wings | |
| | Eosin | Deformed wings with raised tip | |
| | Eosin modification factor | III cross vein | |
| | Sooty | Indentation of inner edge of wings | |
| | Black | | |
| | Aristaless | | |
| | Abnormal abdomen | | |
| | (Eosin stock) | | |
| | Black or Sooty | | |
| | Eosin modification factor | | |
| | Abnormal abdomen | | |
| | (Coral stock) | | |
| | White | | |
| | Black | | |
| | Abnormal addomen | | |

white stock. The crosses resulted in progeny identical with those obtained when the white stock itself is crossed to wild type or to other white stock. It is a significant fact that the abnormal eye condition found in the wild stock appeared in the white progeny of the white males obtained from heat irradiation when mated to white stock females.

One sooty-like female developed directly from the treated larvae, but it died before offspring were obtained. Goldschmidt reported a similar case, for which there is at

present no suitable explanation. Another sooty female was obtained in the $F_2$. It was crossed with wild type and in the succeeding generations behaved as the known stock sooty does, appearing in the heterozygous forms accompanied with a considerably darkened trident pattern on the thorax. The third sooty appeared among the progeny of the treated adults. It likewise showed progeny characteristics of sooty when crossed to wild type.

Sepia was found in three cultures, two in which the larvae were treated and one in which the adults were treated. Of the three females which appeared one was crossed to wild type and the other two to sepia. In the $F_2$ a ratio of three wild type to one sepia was obtained from the wild type cross, and in the cross with sepia all sepia were obtained in the $F_1$ and $F_2$.

The fourth genic modification, abnormal legs, however, could not be identified definitely with any of the known mutations. It is nevertheless an inheritable character appearing in $F_1$, $F_2$ and $F_3$ progeny of inbred abnormal legged individuals. In no case, however, was a pure culture obtainable. This character appears in varying degrees of intensity, the least noticeable cases of abnormal legs showing a crescent-like bending and thickening of the basal tarsi of the hind legs, the crescent points turning either towards or away from the body or parallel to the body. In the extreme cases, besides the bending of the tarsi, there is also a bending in the tibias and in all the five tarsal segments, in all three pairs of legs. Many intermediate forms were observed.

In addition to the above characters found solely in treated flies many hereditable modifications were found to appear in the untreated as well as the treated series. Chief among them was a character restricted to the eye. Such modifications as enlarged eye, indented eye, kidney eye, rough eye, aristaless, reduplicated aristalae, fused facets, papilla-like projections on the eye, bristles completely surrounding the eye, and a partially superim-

posed duplicate eye on either or both eyes, were found in the stock. Numerous irregularities were also found among the dorsocentral and scutellar bristles in both the controls and treated flies. The bristles varied considerably in size and number. Often no bristles appeared on emerging flies, while occasionally as many as five dorsocentral bristles appeared on the newly hatched flies. No mosaics were found among any of the flies examined. Lethals, though probably present, were not identified. Sterility, though stressed by Goldschmidt, appeared definitely in one series only, namely, an exposure of the adults to 39° C. for one hour, when a total of seven flies only were obtained from 3 pairs which were mated individually. All other flies appeared to be normal.

The appearance of twelve mutations among 110,000 flies bred from heat-treated parents, while none appeared among 45,000 untreated flies, indicates that heat treatment greatly increases the rate of mutation. Furthermore, the appearance of half of these mutations (six) among the progeny of heat-treated larvae from 1–2 days old (17,086 flies) apparently indicates that this period in the development of the fly responds to heat treatment at a greater rate than later periods. The mutation rate among the adults was found to be considerably lower, three appearing among 49,317 flies.

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# CULTURE MEDIA FOR DROSOPHILA AND THE pH OF MEDIA

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## PART I. IMPROVEMENTS IN CULTURE METHODS: THE RÔLE OF YEAST

### Gametic and Observed Ratios

In the two decades during which *Drosophila melanogaster* has been bred as material for studies on heredity frequent improvements have been made in the culture media and methods in order to secure unhampered development of the full number of eggs laid by a female during a limited sampling period of ten days or less. This is to obtain the closest possible approximation or parallelism between the directly observed phenotypic series or ratios of zygotes on the one hand and the gametic series or ratios which are the real objects of study. The discrepancy is greatest where relatively weak mutant forms must be raised in competition with wild-type or strong forms. In many classes of experiments the accuracy and importance of the results are directly dependent on absence of distortion in the zygotic ratios, for example, in studies where linkage or non-disjunction is involved. Wherever possible the experiments are planned so as to avoid use of mutant types with high mortality. But often no mutant of normal viability is available for some special region of a chromosome, and of course in all experiments to discover the nature and behavior of those mutant types which happen to have low viability the only resource is to employ optimum culture conditions already

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developed and tested. In testing for the optimum methods these mutants of low viability are good indices, for where weak flies do emerge consistently in the closest approximation to the number of wild-type sibs, there conditions are optimum for both kinds.

### THE FERMENTED BANANA MEDIUM

In the early work, prior to about 1916, fermented banana was generally used as food for the larvae and adults. The usual technique has been published in the 1915 edition of "The Mechanism of Mendelian Heredity" (page 229). The essentials of the method are the following: bananas with intact skins were put into closed jars, as a protection against the chance of egg deposition by stray flies, and were kept there until they became thoroughly ripe. They were then peeled, and the pulp broken into pieces and dropped into another covered jar containing water seeded with yeast. Usually the yeasty liquid used in the jar was the fermented juice from the previous lot of bananas. Fermentation converted the pieces of banana into a spongy mass floating on top of the liquid. The pulp was at its best about a day after starting the fermentation and became too acid for use after the second day. This rather dry fermented pulp was put into the culture bottles on the bottom, in amounts of about 5 grams for each half-pint bottle and was then covered with a layer or two of paper toweling. The flies were next inserted and the bottle stoppered with cotton. If the flies were inserted while still under ether they were first put into a small paper cornucopia which was stuck to the side of the bottle and in which the flies remained safe while recovering.

This amount of food was found not to be sufficient to give unhampered development to all the larvae that could be produced by a mother laying for the usual period of ten days. The flies tended to be small in size, few in number and highly variable in both respects. Preferably a second supply of food was often added, alongside

the first mass so as not to bury any of the eggs or pupae already present. The new food was added after about 5 days. One of the greatest defects of this system was the fact that the jarring necessary in getting the offspring out was very liable to dislodge the food mass, which would then crush or entangle the flies.

### The Alcoholized Banana Medium

In 1915–16 comparative tests were made by Bridges, as the result of which the procedure was improved somewhat (Bridges, 1921). The fermentation was started fresh each time, instead of using the old juice from the last batch of bananas. This delayed the development of acetic acid. The presence of a slight amount of alcohol (optimum 1.5 per cent.) at the beginning of the fermentation was found by test to be conducive to higher outputs. The favorable action of the alcohol is probably exercised in two ways; first, alcohol is a definite stimulant to egg-laying; and second, it is a powerful retarder of the development of competing molds and bacteria. Thus, Baumberger (1919) quotes Lafar (1910, II, p. 238) "From the standpoint of the oecological theory of fermentation, the alcohol produced by yeast should be regarded as a weapon capable of hindering the appearance of other fungoid competitors in saccharine nutrient media. However, when accumulated in the medium during the progress of fermentation, it also restricts the further development of the producer." In this case, as with yeast poisons in general, the first result is the diminution and later the cessation (at about 6 per cent.) of cell reproduction. A still greater concentration reduces fermentation and brings it to a stop (at 14–21 per cent.), while still more alcohol kills the cells. Recently Richards (1928) has studied the inhibiting effect of alcohol on the growth of yeast and on fermentation and finds it very great. But in the usual bacteria-contaminated yeast cultures used for food for flies the alcohol does not accumulate markedly. Instead, it is changed

over by bacterial action into acetic acid. It has long been thought that the increase in acidity of the culture medium is itself a very important obstacle to the culture of flies. The exhaustion of the sugars and yeast foods, from too little or too poor medium, is perhaps the primary factor in limiting the growing of flies.

In the alcohol method the pulp of ripe sound bananas was weighed and put to ferment in a large, shallow, tightly covered glass dish with an equal weight of 3 per cent. alcohol (optimum percentage). Each banana pulp was crushed slightly, little more than enough to separate it into three longitudinal strips. Yeast was added. The optimum amount of drained pulp, fermented 24 hours, for each half pint bottle was found to be 25 grams—more than twice as much as formerly used. Similarly, the optimum amount of paper was 1.0 gram per bottle, instead of about 0.5 gram as formerly used. This paper was used in the form of strips 5 cm long and 0.7 cm wide, matted down on the food, which was placed directly on the bottom. This method was almost entirely free of trouble from the food mass coming loose. No refeeding was necessary. It was found that pint bottles, with a larger area of bottom, gave larger progenies than half-pint bottles, but the greater convenience in handling the smaller bottles led to their adoption as standard. Another innovation was the use of cheesecloth to cover and reinforce the cotton plugs before insertion. The plugs lasted longer and when removed from the bottle did not, as formerly, leave a fringe of cotton fibers which entangled the flies.

The fermented banana and the alcoholized banana media are still occasionally prepared as an emergency measure, or for raising other species of Drosophila, or as a source of food in keeping flies temporarily in vials, or for refeeding old stock or reserve cultures.

### THE BANANA AGAR MEDIUM

In the autumn of 1916, Bridges received from R. W. Glaser directions for making the transparent banana

juice and agar culture medium which he had developed in cooperation with Baumberger (Baumberger and Glaser, 1917) for the clear observation of egg-laying and of the growth and behavior of larvae. Tests were made which showed (Bridges, 1921) that the concentration of food in this medium was inadequate for general use, but that the use of agar to hold the medium in place was a decided improvement over matted absorbent paper. Accordingly, the amount and concentration of food was increased by grinding up and using all the banana pulp instead of using only the clear strained juice. The method was modified and developed until in the spring of 1917 it was generally adopted in the laboratory as the standard method. The details of this method have been published (Bridges, 1921; also in the Laboratory Manual by Morgan, Sturtevant, Muller and Bridges; Henry Holt and Company, 1923), and need only brief comment here. The minimum percentage of agar needed to hold the food fairly solid was determined at roughly 0.7 per cent. One per cent. was adopted as standard, since with increased stiffness of medium there was less loss of pupae by being worked into the food and smothered or drowned. For this same reason considerable paper was used also (about 0.5 gram) and pupation would then generally occur on the paper rather than in the food cake. With this method the risk of contamination of cultures through the food supply was greatly lowered, since one step in the preparation involved heating to a temperature more than sufficient to kill any eggs or larvae which might be present in the bananas before they were run through the potato masher and mixed with the agar solution. The concentration of food in the medium was kept high by using only as much water as weighed banana pulp. The amount of food per bottle was kept high by using 50 cc of the medium, equivalent to the 25 grams of banana pulp previously determined as optimum. This method remained standard in the Columbia laboratories for over ten years and is still widely used elsewhere.

### THE RÔLE OF YEAST

In 1907 E. Guyenot had published an investigation of the nutrition of flies in relation to microorganisms and concluded that for uniformity of results, whether genetic or physiological, the cultures should be entirely free of living microorganisms. Delcourt and Guyenot finally accomplished this aim (1911). They had first used as culture media potatoes or apples, on which many microorganisms flourished. They then transferred flies to fresh culture tubes containing sterilized potato and selected those lines in which the flies had happened to carry over the smallest variety of microorganisms. They thus secured lines from which they had eliminated one by one all microorganisms except living yeast. For a considerable period they carried on their routine cultures of flies satisfactorily on a medium of potato and the pure culture of living yeast that the flies carried over in the transfers. They were of the opinion that the principal food of the flies was the yeast. Finally they used dead sterilized baker's or brewer's yeast held in a mat by absorbent cotton. The stock of nutrient medium consisted of 300 grams of commercial compressed yeast made up to 1,000 cc with water (30 per cent. yeast). About 50 cc of this medium and sufficient cotton to absorb all free water were placed in Ehrlenmeyer flasks and sterilized. The dead yeast proved excellent for the flies but unfavorable for the living yeast. By repeated transfers they were able to secure strains which were entirely free from living yeast, as well as other microorganisms, and which thrived well on the dead yeast medium.

The work of Delcourt and Guyenot was confirmed by Northrop and Loeb (1917) and by Baumberger (1917a). Northrop and Loeb raised flies many generations with success by essentially the same method as that used by Delcourt and Guyenot, *viz.*, upon dead sterilized yeast held in a mat by absorbent cotton. They rendered their flies free from microorganisms by treatment of the eggs

with HgCl, rather than by repeated transfers. Baumberger secured sterile races much more easily by washing pupae or eggs for 10 minutes in 85 per cent. alcohol.

These researches made it clear that normally the yeast growing on the banana or other media, rather than the banana or fruit itself, was the principal food of the larvae, although other microorganisms and the fruit pulp were undoubtedly utilized as well. Baumberger (1917a, 1919) was of the opinion that a loose symbiosis exists in the fact of the universal transference of yeast by eggs, larvae and the feet of adults. The importance of the yeast as food for the larvae lies principally in its high protein count, approximately 11 per cent., whereas the per cent.) that larvae restricted to sterile banana pulp protein concentration in the banana itself is so low (1.3 require two to six times as long to reach pupation, and then only in greatly reduced numbers and of small size (Baumberger, 1919).

Baumberger stated (1917b) that yeast and flies thrived well on Pasteur's nutrient agar medium, the formula for which he reprinted.

The common name of *Drosophila melanogaster* has been "pomace fly," "vinegar fly" "fruit fly," "banana fly," etc., but in the light of these researches it is seen that it should be called the "yeast fly." Another "fruit fly," *Anastrepha ludens,* the Mexican fruit fly, has been reared by Darby (unpublished) on a nutrient medium with yeast as the food. The question may be asked whether all "fruit flies" may not be supported by a diet of microorganisms.

According to Baumberger (1919) those substances tend to attract adult insects and also to induce egg deposition that are the normal products of the activity of the particular microorganism on which the larvae of the insect principally feed. Not "fruity" odors, but odors characteristic of the activity of microorganisms growing on the fruit are the attractions. Hence alcohol derives its attractiveness to Drosophila from the fact that the prin-

cipal normal food of Drosophila larvae is yeast, the alcohol odor being a sign-post to the presence of yeast.

In the commercial growing of yeast, molasses is used widely, and Baumberger (1917b) reported that in raising flies Pasteur's solution could be approximated by diluting molasses with three parts of water. During 1917 Dr. H. J. Muller used a molasses agar medium at Columbia. His best success came from substitution of Karo corn syrup for about half of the molasses content. One may now guess that the improvement lay in reducing the amount of $SO_2$ which was present in the bleached molasses. While this method was fairly successful, he abandoned it presently in favor of the banana agar method that had been improved and standardized.

### PEARL'S SYNTHETIC MEDIA

In connection with his study on population characteristics, Pearl used Drosophila extensively. For uniformity of results he sought to standardize the Drosophila culture medium and to this end experimented with synthetic media, following the lead of Delcourt and Guyenot, of Loeb and Northrop and especially of Baumberger. He reported in a preliminary note (Pearl, 1926) and later in detail (Pearl and Penniman, 1926; Pearl, Allen and Penniman, 1926) on these media. The best medium, S-101, had the following formula:

Solution A

| | |
|---|---|
| Cane sugar | 500 grams. |
| $KNa\ C_4H_4O_6\ 4H_2O$ | 50 " |
| $(NH_4)_2\ SO_4$ | 12 " |
| $Mg\ SO_4\ 7H_2O$ | 3 " |
| $Ca\ Cl_2$ | 15 " |
| $H_2O$ to make 3000 cc of solution. | |

Solution B

| | |
|---|---|
| Agar | 135 grams. |
| Tartaric acid | 30 " |
| $KH_2\ PO_4$ | 6 " |
| $H_2O$ to make 3000 cc of solution. | |

Heat the agar in the water to solution, add the salts, and for the medium to be used in the fly bottles, mix

equal parts of solutions A and B. Use 50 cc per culture. Seed with yeast two days before putting in the parental flies.

The authors reported less mortality of parents and much higher output of progeny on medium S-101 than on the banana agar control.

The experience with this medium in the Columbia laboratory was not as happy as hoped for, and the oral reports of other workers did not indicate that they found it much, if at all, more satisfactory than the banana agar method. Greater trouble with molds was reported.

We are indebted to Mr. W. N. Mertz, working at Park College, Missouri, under the direction of Miss Martha Scott, for the data on which Table 1 is based. These data show that for fertile cultures having the same parentage the average productivity on Pearl's medium S-101 was practically identical with the productivity on banana agar. The number of sterile cultures and the variability in productivity was markedly greater on Pearl's medium. The relative viability of the character white eye was about the same on S-101 as on banana agar, but the viability of males was slightly higher, higher even than the number of females. It is unusual for the number of males to exceed the number of females in an experiment, but on account of the high variability shown not much significance is to be attached to this difference.

### Miscellaneous Media

The use of starch paste as a substitute for agar or paper as binding material was suggested by Dr. C. W. Metz, who had used this method in 1914 and 1915 for raising species other than *Drosophila melanogaster*. In the spring of 1916 considerable work was done by Bridges in testing out various media containing cornstarch, sugar, peptone and salts, with a seeding of yeast. These methods gave high progenies, but trouble with molds was increased.

TABLE 1

COMPARATIVE PRODUCTIVITIES OF SISTER CULTURES INVOLVING RED AND WHITE EYES, SOME REARED ON BANANA AGAR, OTHERS ON PEARL'S S-101 MEDIUM

| Banana agar medium | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | +♀ | +♂ | w ♀ | w ♂ | Av. | % ♂ | % w |
| 24 | 1821 | 1354 | ..... | ..... | 132 | 42.6 | ..... |
| 6 | 355 | 351 | ..... | ..... | 118 | 49.7 | ..... |
| 21 | 1277 | ..... | ..... | 1165 | 116 | 47.7 | 47.7 |
| 8 | ..... | ..... | 462 | 451 | 114 | 49.4 | ..... |
| 23 | 1507 | 693 | ..... | 741 | 128 | 48.8 | 51.6 |
| 11 | 627 | 445 | ..... | 319 | 126 | 54.9 | 41.7 |
| 23 | 747 | 752 | 733 | 691 | 127 | 49.4 | 48.7 |
| 6 | 208 | 230 | 192 | 195 | 137 | 51.5 | 46.9 |
| 122 | | 15,316 | | | 126 | 48.2 | 48.1 |

| Pearl's S-101 medium | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | +♀ | +♂ | w ♀ | w ♂ | Av. | % ♂ | % w |
| 2 | 129 | 100 | ..... | ..... | 115 | 43.6 | ..... |
| 4 | 221 | 286 | ..... | ..... | 127 | 56.4 | ..... |
| 7 | 382 | ..... | ..... | 443 | 118 | 53.7 | 57.7 |
| 4 | ..... | ..... | 165 | 185 | 87 | 52.8 | ..... |
| 8 | 603 | 381 | ..... | 275 | 157 | 52.1 | 41.9 |
| 3 | 156 | 119 | ..... | 74 | 116 | 55.3 | 38.3 |
| 7 | 249 | 274 | 271 | 270 | 152 | 50.8 | 51.1 |
| 4 | 91 | 107 | 99 | 101 | 100 | 52.3 | 50.2 |
| 39 | | 4,981 | | | 128 | 52.4 | 48.9 |

For his work on the distribution of crossing-over in the third chromosome of *Drosophila melanogaster* Gowen (1919) used fermented banana during 1915 and early 1916, but changed in June, 1916, to an artificial food mixture of starch, sugar, peptone, yeast and water. The crossing-over results were unaffected by the change in food material.

Dr. O. L. Mohr, in working in Norway, where bananas were rare, tried various other fruits and was of the opinion in 1920 that dried pear was the most convenient and satisfactory substitute for banana.

Dr. Th. Dobzhansky relates that in Russia he used a raisin agar medium, developed prior to 1923, in the laboratory of Chetverikov. This medium was prepared from 100 parts water, 50 parts raisins, ground very fine by a meat chopper, and 2 to 3 parts agar. This medium has the advantage that molds rarely grow upon it, especially if yeast is added.

Later, on account of the scarcity of agar, the Russian workers have added potato pulp to their media, but at the expense of greater trouble with molds. The formula given by Gershenson (1928) for use with *D. obscura* is: water 800, fermented raisins 100, cooked mashed potatoes 400, agar 4.

Komai (1927) reported on a synthetic medium developed by his coworker, M. Chino. The formula was:

| | |
|---|---|
| Peeled banana pulp | 100 gm. |
| Kôji | 100 gm. |
| Water | 800 gm. |

Kôji was described as a culture of *Aspergillus oryzae* on rice, commonly used in Japan for fermenting rice for brewing sake and for other purposes. The kôji supplied protein and was relatively inexpensive in Japan. Komai recommended 34 gm per half-pint culture bottle as compared with the 50 gm of banana agar medium generally used. This medium he found to be cheaper than the banana agar medium, as satisfactory in the rearing of *Drosophila melanogaster* and apparently better for *D. virilis*.

## PART II. CORNMEAL MOLASSES AGAR MEDIA

### Medium A

After ten years' use the banana agar method became somewhat unsatisfactory from the standpoint of relative cost. The price of bananas was becoming higher and the quantity used was increasing rapidly. The texture of the banana agar medium was also not too satisfactory, in that it was not fibrous or porous, as was the case with

the fermented banana and paper medium. This defect resulted in surface action of the larvae rather than penetration by the larvae through and through the mass. The use of paper helped, but the paper did not mix with the agar thoroughly enough. Bran or oat hulls seemed better, but bran was thought to increase mold and oat hulls were hard to get.

In the spring of 1926, Dr. Helen Redfield tried a number of combinations with molasses as the food and various materials as fillers and stiffeners. All these yielded offspring. They included bran and molasses, agar and molasses, agar and bran and molasses, cooked cornmeal and molasses, cooked cornmeal bran and molasses, cooked cornmeal agar and molasses. It was hoped to develop a formula excluding the expensive agar. The boiled cornmeal medium, without agar, formed a starch paste stiff enough to stand the jarring of the cultures, provided enough cornmeal was used (20 per cent.). But late in the course of the cultures there was a softening of the paste that was troublesome. Also the medium was so viscous that in the process of preparation it was very difficult to put it into the culture bottles. By combination with agar these difficulties were overcome and the percentage of cornmeal reduced. As the source of sugar a mixture of "Brer Rabbit" brand of molasses and Karo corn syrup, as originally developed by Muller, was used. Redfield's formula follows:

For each 100 cc of the medium:

| | |
|---|---|
| Water | 62.5 cc. |
| Agar | 0.8 gm. |
| Cornmeal ("Quaker Oats") | 16.7 gm. |
| Molasses | 11.0 cc. |
| Karo corn syrup | 9.0 cc. |

Two thirds of the water was used to dissolve the agar; the molasses was then added. The meal, previously moistened with a third of the water to prevent lumping, was stirred in and the mixture cooked until it stiffened. This food has been used by Redfield with satisfactory results for *Drosophila melanogaster, D. obscura,*

*D. simulans, D. hydei, D. immigrans* and *D. repleta.* For *D. virilis,* however, it seems not to be as successful as the old banana-agar medium.

For general laboratory use it was thought that the expense of the medium might be further reduced by using a cheap grade of chicken-feed cornmeal. In using this meal in the summer of 1926 Bridges found that the formula should be modified as follows:

| For each 100 cc of the medium: | | |
|---|---|---|
| | Water | 68 cc. |
| | Agar | 1 gm. |
| Medium A. | Cornmeal | 16 gm. |
| | Molasses | 8 cc. |
| | Karo corn syrup | 7 cc. |

In preparing this medium, about a quarter of the water was saved to be added as the last step, in order to thin the stiffened cornmeal to a consistency that could be poured without too much trouble. For pouring, the thinned medium was put into a very large funnel supported by a ring stand. The stem of the funnel was terminated with a large thin-walled rubber tube and the pouring was controlled by a large spring clamp on the tube. About 50 cc was used per half-pint bottle, with toweling paper and yeast on top.

## The pH Changes of Media

Pearl had been of the opinion that a high H-ion concentration (pH 3.0) was favorable to flies because unfavorable to troublesome bacteria and molds. He had investigated the pH changes in the banana agar, and in his medium S-99, rather carefully.

Some work by Darby (1930) had shown that the optimum pH for the growth of yeast is at approximately pH 4.45. The question arose: What pH did the new medium show and could this be modified in a favorable direction.

### The Spot Method of Colorimetric Determination of pH

The technique employed by Darby in making these determinations was essentially the "spot" method of

Felton (1921) with a few modifications for the sake of higher speed in carrying out large numbers of determinations. Since the values obtained at the end regions of the ranges of the various indicators are uncertain, the determinations have been made in duplicate wherever it was possible to check by a second determination with an indicator of overlapping range.

### THE pH OF MEDIUM A, WITHOUT FLIES

Ten culture bottles were made up, on April 9, 1927, according to the formula just given, using 50 cc of medium A per bottle. The cultures were seeded with

TABLE 2

MEDIUM A, WITHOUT FLIES; pH READINGS ON SUCCESSIVE DAYS

| No. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.7 | 5.8 | 6.0 | 5.2 | 4.0 | 3.7 | 3.7 | 3.7 | 3.7 |
| 2 | 5.7 | 5.7 | 5.4 | 5.2 | 4.4 | 3.9 | 3.9 | 3.8 | 3.7 |
| 3 | 5.7 | 5.7 | 5.4 | 4.8 | 3.8 | 3.7 | 3.7 | 3.7 | 3.7 |
| 4 | 5.7 | 5.6 | 5.4 | 5.2 | 4.0 | 3.7 | 3.7 | 3.7 | 3.8 |
| 5 | 5.7 | 5.7 | 5.4 | 4.8 | 4.0 | 3.6 | 3.6 | 3.6 | 3.6 |
| 6 | 5.7 | 5.8 | 6.0 | 5.2 | 4.2 | 3.7 | 3.7 | 3.7 | 3.7 |
| 7 | 5.7 | 5.7 | 5.3 | 4.9 | 3.8 | 3.7 | 3.7 | 3.7 | 3.7 |
| 8 | 5.7 | 5.7 | 5.5 | 5.2 | 4.3 | 3.7 | 3.7 | 3.7 | 3.7 |
| 9 | 5.7 | 5.7 | 5.3 | 5.1 | 4.2 | 3.9 | 3.8 | 3.8 | 3.8 |
| 10 | 5.7 | 5.8 | 5.9 | 4.6 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 |
| Av. pH | 5.7 | 5.7 | 5.6 | 5.0 | 4.0 | 3.7 | 3.7 | 3.7 | 3.7 |

yeast, in the usual manner, but no flies were put in. The bottles were kept in an incubator at 25° C. A determination of the pH was made shortly after the bottles were cool and at 24-hour intervals thereafter for eight days. The results are given in Table 2. The initial determinations of pH, made just after the bottles were cool, gave the same reading, 5.7 for all cultures. This was slightly higher than the initial reading of 5.3 found for banana agar by Pearl and Penniman and confirmed by Darby. At the end of 24 hours no change had occurred. By the end of 48 hours the pH had dropped

very slightly to an average of 5.6. At the end of three days the average pH had dropped to 5.0 and during the next day, the fourth, it made a very large drop to 4.0. By the end of the fifth day it had fallen slightly more to 3.7, a value which was maintained to the termination of the observations at the end of the eighth day.

It is to be noted that the degree of resemblance between the different cultures was very high indeed throughout the course of the observations.

In five cultures (2, 5, 7, 8, 9) mold developed, but this apparently made no difference. The lowest final reading (3.6) and the highest final reading (3.8) occurred in two of the moldy bottles.

### The pH of Medium A, with Flies

The effect of the presence of flies and developing larvae on the pH of the culture medium was investigated. Twelve culture bottles were made up containing 50 cc of medium A. As soon as they were cool a pair of 3-day-old flies were put in each, and the cultures were incubated at 25° C. The determinations were made as before, but the course of the cultures was followed for a longer time, for eighteen days altogether. The parental flies were left in the cultures during the first ten days. The flies of the offspring generation were removed from the cultures as they emerged, but were not counted.

As shown by the means in Table 3, the pH of the bottles with flies began to drop at once and reached the pH levels of 5, of 4.5 and of 4, respectively, about a day and a half sooner than the cultures without flies. The low level reached and maintained was 3.7, exactly as in the case of the cultures without flies. But by the end of the 10th day the pH had risen slightly to 4, and at the end of eighteen days it had reached the value 4.8, by a slow graded rise.

Since the difference in pH early in the cultures had been determined from experiments run successively rather than simultaneously, and since the second experiment had shown a pH rise in the cultures at a period

TABLE 3

MEDIUM A, WITH FLIES; pH READINGS ON SUCCESSIVE DAYS

| No. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 10 | 14 | 16 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.8 | 5.8 | 4.7 | 3.8 | 3.7 | 3.7 | 3.7 | 3.8 | 4.4 | 4.8 | 4.9 | 4.8 |
| 2 | 5.8 | 5.6 | 4.7 | 3.9 | 3.6 | 3.6 | 3.6 | 3.6 | 3.8 | 4.8 | 5.0 | 4.8 |
| 3 | 5.8 | 5.3 | 4.6 | 4.0 | 3.8 | 3.7 | 3.6 | 3.7 | 3.9 | 4.8 | 5.0 | 4.8 |
| 4 | 5.8 | 5.6 | 4.4 | 3.7 | 3.5 | 3.5 | 3.5 | 3.5 | 3.7 | 4.2 | 4.4 | 4.6 |
| 5 | 5.8 | 5.6 | 4.6 | 3.9 | 3.8 | 3.8 | 3.8 | 3.8 | 4.6 | 4.8 | 4.8 | 4.8 |
| 6 | 5.8 | 5.6 | 4.4 | 3.7 | 3.5 | 3.4 | 3.4 | 3.6 | 3.6 | 4.1 | 4.2 | 5.0 |
| 7 | 5.8 | 5.7 | 4.7 | 3.9 | 3.8 | 3.7 | 3.7 | 3.7 | 4.4 | 4.4 | 4.6 | 4.8 |
| 8 | 5.8 | 5.5 | 4.6 | 3.8 | 3.7 | 3.7 | 3.7 | 3.8 | 4.0 | 4.5 | 4.9 | 4.8 |
| 9 | 5.8 | 5.5 | 4.0 | 3.6 | 3.6 | 3.7 | 3.7 | 4.0 | 4.0 | 4.1 | 4.1 | 4.6 |
| 10 | 5.8 | 5.8 | 4.6 | 3.9 | 3.6 | 3.8 | 3.9 | 4.0 | 4.1 | 4.8 | 5.0 | 4.6 |
| 11 | 5.8 | 5.6 | 4.5 | 3.8 | 3.7 | 3.6 | 3.6 | 3.8 | 3.8 | 4.6 | 4.7 | 4.9 |
| 12 | 5.8 | 5.6 | 4.6 | 3.8 | 3.6 | 3.6 | 3.6 | 3.6 | 3.8 | 4.2 | 4.4 | 4.6 |
| Av. pH | 5.8 | 5.6 | 4.5 | 3.8 | 3.7 | 3.7 | 3.7 | 3.7 | 4.0 | 4.5 | 4.7 | 4.8 |

later than was covered by the first experiment, it seemed advisable to run a small-scale experiment to check these two points.

The cultures were made as before, using 50 cc of medium A. In half of the bottles pairs of 3-day old flies were put and were allowed to remain 5 days only.

As shown by means of Table 4 the fall through succes-

TABLE 4

MEDIUM A; WITHOUT FLIES, CULTURES 1–4; WITH FLIES, 5–8; pH READINGS ON SUCCESSIVE DAYS

| No. | 0 | 1 | 2 | 3 | 4 | 6 | 8 | 10 | 12 | 14 | Flies |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.7 | 5.7 | 5.4 | 5.1 | 4.0 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 | ..... |
| 2 | 5.7 | 5.8 | 6.0 | 5.4 | 4.6 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | ..... |
| 3 | 5.7 | 5.7 | 5.6 | 4.3 | 3.6 | 3.8 | 3.9 | 4.0 | 4.0 | 4.0 | ..... |
| 4 | 5.7 | 5.7 | 5.6 | 5.0 | 4.3 | 3.7 | 3.7 | 3.7 | 4.2 | 4.2 | ..... |
| Av. | 5.7 | 5.7 | 5.7 | 5.0 | 4.1 | 3.7 | 3.7 | 3.8 | 3.9 | 3.9 | ..... |
| 5 | 5.7 | 5.4 | 4.6 | 3.9 | 3.6 | 3.6 | 3.7 | 4.2 | 4.2 | 4.4 | 38 |
| 6 | 5.7 | 5.5 | 4.5 | 3.8 | 3.5 | 3.5 | 3.5 | 3.6 | 3.6 | ..... | 60 |
| 7 | 5.7 | 5.6 | 5.0 | 4.0 | 3.6 | 3.6 | 3.7 | 3.7 | 3.6 | 3.6 | 91 |
| 8 | 5.7 | 5.6 | 5.3 | 4.5 | 3.6 | 3.6 | 3.6 | 3.8 | 3.8 | 4.0 | 95 |
| Av. | 5.7 | 5.5 | 4.9 | 4.1 | 3.6 | 3.6 | 3.6 | 3.8 | 3.8 | 4.0 | 71 |

sive pH values to the minimum pH occurred slightly more than a day earlier in the cultures containing flies than in the cultures free from flies. Also the slight rise late in the cultures occurred in the cultures free from flies as well as in the cultures with flies.

The rightmost column of Table 4 gives the number of flies that hatched during a 4-day count of the progeny.

## Medium A, with $KH_2PO_4$

The effect produced upon medium A by the addition of primary potassium phosphate was tested as follows: Cultures 1 to 10, Table 5, were made as a control, using

TABLE 5

Medium A; Cultures 1–10; without Flies; Cultures 11–16, without Flies, with $KH_2PO_4$; Cultures 16–25, with Flies, with $KH_2PO_4$

| No. | 1 | 2 | 3 | 4 | 5 | 7 | 9 | 11 | 13 | 16 | 18 | 20 | Flies |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.9 | 4.2 | 4.0 | 3.8 | 3.6 | 3.8 | 4.3 | 4.4 | 4.4 | 4.3 | 3.8 | ..... | ..... |
| 2 | 6.0 | 4.1 | 3.9 | 3.6 | 3.6 | 3.8 | 4.0 | 4.1 | 4.3 | 4.2 | 4.2 | ..... | ..... |
| 3 | 5.9 | 4.0 | 4.0 | 3.9 | 3.6 | 4.1 | 4.1 | 4.2 | 4.2 | 4.4 | 4.5 | ..... | ..... |
| 4 | 6.0 | 4.0 | 4.0 | 3.8 | 3.9 | 3.9 | 4.1 | 4.3 | 4.3 | 4.3 | 4.3 | ..... | ..... |
| 5 | 6.0 | 4.1 | 4.0 | 3.8 | 3.8 | 3.8 | 4.2 | 4.0 | 4.2 | 3.8 | 4.2 | ..... | ..... |
| 6 | 6.0 | 4.2 | 4.0 | 3.6 | 3.8 | 3.8 | 4.1 | 4.1 | 4.4 | 4.1 | 4.4 | ..... | ..... |
| 7 | 5.9 | 4.1 | 3.9 | 3.8 | 3.8 | 4.0 | 4.1 | 4.0 | 4.2 | 4.2 | 4.3 | ..... | ..... |
| 8 | 6.0 | 4.3 | 4.2 | 4.0 | 3.7 | 3.8 | 3.9 | 4.4 | 4.4 | 4.1 | 4.1 | ..... | ..... |
| 9 | 5.9 | 4.1 | 3.9 | 3.7 | 3.8 | 3.8 | 4.1 | 4.2 | 4.1 | 4.0 | 4.2 | ..... | ..... |
| 10 | 5.9 | 4.0 | 4.0 | 3.8 | 3.7 | 3.7 | 4.0 | 4.2 | 4.2 | 4.2 | 4.1 | ..... | ..... |
| Av. | 6.0 | 4.1 | 4.0 | 3.8 | 3.7 | 3.9 | 4.1 | 4.2 | 4.3 | 4.2 | 4.2 | ..... | ..... |
| 11 | 5.4 | 4.0 | 3.7 | 3.7 | 3.8 | 4.0 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | ..... | ..... |
| 12 | 5.5 | 4.0 | 3.8 | 3.8 | 3.8 | 4.4 | 4.2 | 4.1 | 4.1 | 4.1 | 4.1 | ..... | ..... |
| 13 | 5.4 | 3.9 | 3.8 | 3.8 | 3.8 | 4.1 | 4.4 | 4.1 | 4.1 | 4.1 | 4.1 | ..... | ..... |
| 14 | 5.5 | 4.0 | 3.8 | 3.7 | 3.7 | 4.2 | 4.3 | 4.2 | 4.2 | 4.1 | 4.1 | ..... | ..... |
| 15 | 5.4 | 4.0 | 3.8 | 3.8 | 3.8 | 4.2 | 4.2 | 4.2 | 4.1 | 4.1 | 4.1 | ..... | ..... |
| Av. | 5.4 | 4.0 | 3.8 | 3.8 | 3.8 | 4.2 | 4.2 | 4.1 | 4.1 | 4.1 | 4.1 | ..... | ..... |
| 16 | 6.0 | 4.1 | 4.0 | 3.7 | 4.0 | 4.0 | 4.0 | 3.9 | 3.9 | 4.0 | 4.0 | 4.0 | 141 |
| 17 | 6.0 | 4.0 | 4.0 | 3.8 | 3.8 | 4.0 | 4.0 | 3.9 | 4.0 | 4.2 | 4.1 | 4.2 | 165 |
| 18 | 5.9 | 4.1 | 4.0 | 3.7 | 3.7 | 4.0 | 4.0 | 3.9 | 4.0 | 4.0 | 4.0 | 4.1 | 151 |
| 19 | 5.9 | 4.2 | 3.9 | 3.8 | 3.9 | 3.9 | 4.0 | 3.9 | 4.0 | 3.9 | 4.1 | 4.1 | 186 |
| 20 | 6.0 | 4.1 | 4.0 | 3.7 | 3.7 | 3.8 | 3.9 | 3.8 | 4.0 | 3.8 | 4.0 | 4.0 | 223 |
| 21 | 6.0 | 4.0 | 3.9 | 3.8 | 3.6 | 4.0 | 3.8 | 3.8 | 3.9 | 3.9 | 4.0 | 4.0 | 303 |
| 22 | 5.9 | 4.0 | 4.0 | 3.8 | 3.8 | 3.9 | 4.1 | 3.9 | 4.0 | 4.1 | 4.3 | 4.3 | 189 |
| 23 | 6.0 | 4.1 | 3.8 | 3.6 | 3.8 | 4.0 | 4.1 | 4.0 | 4.0 | 4.0 | 4.1 | 4.0 | 151 |
| 24 | 6.0 | 4.0 | 4.0 | 3.7 | 3.8 | 4.0 | 4.1 | 4.1 | 4.2 | 4.1 | 4.4 | 4.2 | 124 |
| 25 | 6.0 | 4.1 | 4.0 | 3.7 | 3.6 | 4.1 | 4.1 | 4.1 | 4.2 | 4.2 | 4.1 | 4.0 | 89 |
| Av. | 6.0 | 4.1 | 4.0 | 3.7 | 3.8 | 4.0 | 4.0 | 3.9 | 4.0 | 4.0 | 4.1 | 4.1 | 172 |

unmodified medium A, without flies. As a second control, cultures 11–15 were run simultaneously, without flies but with the addition of 0.2 gm $KH_2PO_4$ per culture. Finally, in cultures 16–25, run simultaneously with the others, medium A, modified by the addition of 0.2 gm $KH_2PO_4$ per culture, was used with pairs of three-day old flies from the Florida wild stock. Eggs were laid for nine days and the emerging offspring were counted for ten days only. The pH determinations were continued relatively long, for eighteen or twenty days.

Within each set in Table 5 the variability in pH from culture to culture was small. The most striking fact about the three sets is their remarkable similarity to each other. The only difference made by the relatively small amount of phosphate would seem to have been a slight lowering of the initial pH. In contrast to the results in the previous two experiments, the set without flies showed as rapid a drop in pH as did the set with flies. Moreover, all three curves fell to approximately the minimum pH in about two days instead of the three to five days shown previously. The specific factor that differed in the preparation of the early and later cultures is not known. It might be any factor that gave the yeast an unusually good start, such as being more thoroughly distributed over the surface instead of being applied as a single drop. It seemed possible that the difference found earlier between the cultures with and without flies was a result of the rapid distribution of yeast by the feet of the flies and by the crawling young larvae.

The slight rise in pH following the first minimum was present in all three sets. It may be surmised that this rise was due to the production of ammonia or other waste products of alkaline tendency by the growing yeasts and larvae.

The number of offspring in the phosphated cultures averaged 172, or if culture 25 be omitted (abnormal in that flies began emerging several days later than in the others), 182. This value of 182 flies per culture seemed

higher than the average for normal unphosphated cultures, as seen in routine work.

### Effect of Phosphate on Productivity, Medium A

The buffering effect of the phosphate on the pH of the medium had been negligible in this pH range and in the amount used. But phosphates have other effects, such as modifying sugar metabolism, and it was thought such effects had been important here. Accordingly, two sets of medium-A cultures were run simultaneously, both with three-day flies from Florida wild stock. One set, cultures 1–9, Table 6, served as control and the other cultures (10–18) were modified by the additions of 0.2 gm $KH_2PO_4$ per culture. The parents remained in the bottles for nine days, and the counts of the offspring were continued for nine days only. The parents in cultures

TABLE 6

Cultures 1–9, Medium A; 10–18, Medium A, with $KH_2PO_4$

| No. | 1 | 3 | 5 | 9 | 11 | 13 | 15 | Flies |
|---|---|---|---|---|---|---|---|---|
| 1 | 5.5 | 4.4 | 3.6 | 3.6 | 3.8 | 4.1 | 4.3 | 125 |
| 2 | 5.6 | 4.4 | 3.6 | 3.6 | 3.6 | 3.6 | 3.8 | 165 |
| 3 | 5.6 | 4.4 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 136 |
| 4 | 5.6 | 4.7 | 3.6 | 3.7 | 3.6 | 3.6 | 3.7 | 212 |
| 5 | 5.6 | 4.1 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 170 |
| 6 | 5.6 | 4.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 131 |
| 7 | 5.7 | 4.4 | 3.7 | 3.7 | 3.8 | 4.0 | 4.0 | 83 |
| 8 | 5.6 | 4.4 | 3.6 | 3.8 | 3.7 | 4.0 | 4.0 | 119 |
| 9 | 5.6 | 4.5 | 3.6 | 3.7 | 3.7 | 3.6 | 3.6 | 124 |
| Av. | 5.6 | 4.5 | 3.6 | 3.7 | 3.7 | 3.8 | 3.9 | 142 |
| 10 | 5.5 | 3.7 | 3.6 | 3.6 | 3.8 | 3.8 | 3.8 | 267 |
| 11 | 5.6 | 3.7 | 3.7 | 3.8 | 3.8 | 3.8 | 3.8 | 241 |
| 12 | 5.5 | 3.7 | 3.6 | 3.6 | 3.8 | 4.0 | 3.9 | 215 |
| 13 | 5.4 | 3.6 | 3.5 | 3.6 | 3.7 | 3.7 | 3.8 | 284 |
| 14 | 5.5 | 3.9 | 3.7 | 3.6 | 3.7 | 3.8 | 3.8 | 228 |
| 15 | 5.5 | 3.7 | 3.7 | 3.9 | 3.9 | 4.0 | 4.0 | 198 |
| 16 | 5.4 | 3.7 | 3.3 | 3.8 | 3.8 | 3.8 | 3.8 | 229 |
| 17 | 5.5 | 3.9 | 3.7 | 3.9 | 3.7 | 3.8 | 3.8 | 183 |
| 18 | 5.4 | 3.9 | 3.7 | 3.8 | 4.0 | 4.0 | 4.0 | 212 |
| Av. | 5.5 | 3.7 | 3.6 | 3.7 | 3.8 | 3.9 | 3.9 | 229 |

1 and 7 died early. The average progeny for the remaining seven normal cultures was 151. The average number of offspring in the cultures modified by the addition of phosphate was 229, or half again as many in the control cultures. The presence of the phosphate seems therefore to have had again a favorable effect upon the productivity, and to have decreased variability.

The curve for the means of the pH determinations of the buffered cultures is practically identical with that of the control cultures, except for the lowered pH value early in the course of the experiment.

### SOME DEVELOPMENTS OF THE CORNMEAL MOLASSES AGAR METHOD

"Brer Rabbit" molasses, in common with nearly all the commercial brands of molasses easily available, was characterized by the presence of $SO_2$. The $SO_2$ may have been used to make the color lighter, or may have been used to check the fermentation that occurs in the less heavy grades of molasses. That this $SO_2$ would likewise check the growth of yeast in the culture bottles seemed likely. Accordingly, a supply of molasses of high sugar content and entirely free from $SO_2$ was secured from a wholesaler.

In testing this heavy molasses as a substitute of the former mixture of molasses and "Karo" the amount was decreased, since it seemed that the old formula was unnecessarily high in carbohydrate and relatively low in nitrogen supply. The cornmeal was increased slightly to augment the nitrogen but could not be increased much more without making the medium crumbly under the action of the larvae. The first formula tried was the following:

| | | |
|---|---|---|
| Medium B | Water | 70.0 cc. |
| | Agar | 1.2 gm. |
| | Cornmeal | 17.0 gm. |
| | Molasses | 11.8 cc. |

Seven cultures made up according to this formula (Table 7) gave pH readings more favorable than the old formula in that the minimum was about 4.0 rather than 3.7, and was consequently nearer the optimum pH for yeast growth, namely, 4.5. The parents (all experiments employed the Florida stock) were left in the cultures for a ten-day laying period. The offspring, counted for ten days of emergence, averaged 266, higher than the 151 of medium A and than the 182 and 229 of phosphated medium A. Part of this increase was presumably due to the fact that the amount of media per culture was increased from 50 cc to 60 cc. The cultures were entirely free from molds, and in general characteristics seemed preferable to those using the older formula.

TABLE 7
MEDIUM B

| No. | Days | | | Flies |
|---|---|---|---|---|
| | 1 | 3 | 7 | |
| 1 | 5.8 | 4.9 | 4.0 | 191 |
| 2 | 5.7 | 4.9 | 4.0 | 292 |
| 3 | 5.8 | 5.0 | 4.1 | 273 |
| 4 | 5.8 | 4.9 | 4.0 | 334 |
| 5 | 5.8 | 5.0 | 3.9 | 320 |
| 6 | 5.7 | 4.9 | 4.1 | 272 |
| 7 | 5.8 | 5.0 | 4.0 | 277 |
| Av. | 5.8 | 4.9 | 4.0 | 266 |

TABLE 8
MODIFIED MEDIUM B

| No. | 1 | 3 | 7 | Flies |
|---|---|---|---|---|
| 1 | 4.9 | 4.3 | 4.3 | 208 |
| 2 | 4.9 | 4.3 | 4.4 | ..... |
| 3 | 5.0 | 4.4 | 4.4 | 33 |
| 4 | 4.9 | 4.3 | 4.2 | 341 |
| 5 | 4.9 | 4.3 | 4.4 | 144 |
| 6 | 5.0 | 4.3 | 4.3 | ..... |
| 7 | 4.9 | 4.4 | 4.4 | 289 |
| 8 | 4.9 | 4.3 | 4.3 | 358 |
| 9 | 5.0 | 4.3 | 4.4 | 464 |
| Av. | 4.9 | 4.3 | 4.3 | 204 |

### MEDIUM B, MODIFIED BY TARTRATE AND PHOSPHATE

The culture media used for yeasts are usually provided with nitrogen in the form of an ammonium salt. Tartrates are also used and have a buffering action. We therefore ran a test series (Table 8) in which culture medium B was modified by the substitution of one gram of ammonium tartrate for one gram of the cornmeal. A small amount (0.1 gm per hundred parts) of $KH_2PO_4$ was also added.

The pH was initially relatively low (4.9) and its minimum was relatively high (4.3), both deviations being in the direction of stabilizing at the optimum pH for yeast growth. There was an exceptionally heavy growth of yeast in all the cultures. But the production of offspring was very erratic, both in totals and in time of emergence. Two of the cultures produced no flies, although very large numbers of small larvae had been present and many had pupated as small pupae. In another culture 33 small pale flies were produced from large numbers of small larvae and pupae. On the other hand, three cultures produced the unusually large number of about 300 flies each and one produced 464 flies, although these also were rather small and pale. It was supposed that the ammonium tartrate had been used in too great a concentration and had exercised an unfavorable direct effect upon the larvae in addition to the apparently highly favorable effect upon the yeast.

### MEDIUM C, PLAIN AND MODIFIED

On account of the great irregularity in the above result, the test was repeated, but with slight changes in the formula. The amount of ammonium tartrate was reduced to half the previous amount (to 0.5 gm per 100 parts of the medium). "Quaker Oats" cornmeal was used in medium C instead of the usual chicken-feed cornmeal. "Quaker Oats" cornmeal, intended for use as a breakfast cereal, has been very carefully sifted as to size of granule and portion of the grain used and is a much more uniform product than the ordinary cornmeal, which is the mixture of various sized granules and flourlike dust which comes directly from the grinders. "Quaker Oats" cornmeal makes a much stiffer starch paste when it sets, and accordingly the percentage of agar was lowered to 1 instead of the 1.2 that had been used in the former series.

The nine cultures using plain medium C (Table 9) showed an initial pH of 5.8 and a minimum apparently

not far from the optimum of 4.5. The cultures uniformly produced very high outputs, averaging 308, of large flies. The parents had been in nine days, and the offspring were counted for ten days' emergence.

In the nine cultures modified by the ammonium tartrate and the phosphate (Table 10) the pH was apparently the same as in the plain medium C. The output averaged practically the same (296), but most of the cultures were slightly lower, while two had unusually high progenies of over 500 flies. Hence the results of the modification with ammonium tartrate were again irregular, but showed the possibility of marked benefit.

### Medium D and Some Modifications

Some tests just completed by Mr. M. Harnley (1929) on the banana agar medium had shown that greater numbers of offspring were produced when the amount of agar was increased above the 1.0 that had been standard for years. Accordingly, in the next set of tests, in November, 1927, the agar concentration was raised to 1.4 per cent. Four series of cultures with flies were run, using the medium with higher agar content (medium D) and some modifications of it.

| | D | D-1 | D-2 | D-3 |
|---|---|---|---|---|
| Water ..... (cc) | 70.0 | 70.0 | 70.0 | 70.0 |
| Agar ..... (gm) | 1.4 | 1.4 | 1.4 | 1.4 |
| Cornmeal ..... (gm) | 17.0 | 17.0 | 17.0 | 17.0 |
| Molasses ..... (cc) | 11.6 | 11.5 | 10.6 | 11.1 |
| $KH_4PO_2$ ..... (gm) | ..... | 0.1 | ..... | ..... |
| $(NH_4)_2C_4H_4O_6$ (gm) | ..... | ..... | 1.0 | ..... |
| $HNO_3$ ..... (gm) | ..... | ..... | ..... | 0.5 |
| Total parts ..... | 100.0 | 100.0 | 100.0 | 100.0 |

In Table 11 are given the results of the 10 cultures in which medium D was employed. Great care was used to have the different cultures precisely alike initially. The pH was uniformly 5.8 after cooling and 5.7 at the end of one day. The fall was rapid on the second and third

TABLE 9
MEDIUM C

| No. | 0 | 1 | 2 | 3 | 4 | 9 | Flies |
|---|---|---|---|---|---|---|---|
| 1 | 5.7 | 5.7 | 5.2 | 4.6 | 4.5 | 4.0 | 352 |
| 2 | 5.8 | 5.7 | 5.2 | 4.6 | ..... | ..... | 350 |
| 3 | 5.8 | 5.7 | 5.0 | 4.5 | ..... | 4.0 | 221 |
| 4 | 5.8 | 5.7 | 5.1 | 4.8 | 4.8 | ..... | 296 |
| 5 | 5.8 | 5.7 | 5.2 | 4.5 | ..... | ..... | 283 |
| 6 | 5.8 | 5.6 | 5.2 | 4.6 | 4.8 | 4.4 | 346 |
| 7 | 5.8 | 5.7 | 5.2 | 4.5 | 4.6 | ..... | 271 |
| 8 | 5.8 | 5.7 | 5.2 | 4.3 | ..... | ..... | 264 |
| 9 | 5.8 | 5.7 | 5.2 | 4.6 | 4.6 | 4.1 | 390 |
| Av. | 5.8 | 5.7 | 5.2 | 4.6 | 4.6 | 4.1 | 308 |

TABLE 10
MEDIUM C, MODIFIED

| No. | 0 | 1 | 2 | 3 | 4 | 9 | Flies |
|---|---|---|---|---|---|---|---|
| 1 | 5.8 | 5.7 | 4.3 | ..... | 4.2 | 4.3 | 507 |
| 2 | 5.8 | 5.6 | 4.4 | ..... | ..... | ..... | 252 |
| 3 | 5.8 | 5.7 | ..... | 4.4 | ..... | ..... | 243 |
| 4 | 5.8 | 5.7 | 4.6 | 4.6 | 4.6 | 4.6 | 191 |
| 5 | 5.8 | 5.6 | ..... | ..... | 4.2 | ..... | 213 |
| 6 | ..... | 5.7 | ..... | ..... | ..... | ..... | 228 |
| 7 | 5.8 | 5.6 | ..... | ..... | 4.0 | 4.0 | 258 |
| 8 | 5.8 | 5.7 | ..... | 4.4 | 4.1 | ..... | 559 |
| 9 | 5.8 | 5.7 | 4.6 | ..... | ..... | 4.1 | 215 |
| Av. | 5.8 | 5.7 | 4.5 | 4.5 | 4.2 | 4.2 | 296 |

days, to the minimum of 4.1 on the fourth day. The readings were not continued after it was seen by the fifth day's results that the minimum had been reached.

Into each bottle was put, shortly after the cooling and the initial determination of the pH, a pair of two-day-old flies from the Florida wild stock. They were allowed to remain six days. Only in the case of culture 3 had the female perished some time before the removal. The offspring were counted daily as they emerged. It had been intended to continue the counts for ten days, but after eight days of counting the heat control in the incubator

TABLE 11
MEDIUM D

| No. | 0 | 1 | 2 | 3 | 4 | 5 | Flies |
|---|---|---|---|---|---|---|---|
| 1 | 5.8 | 5.7 | 5.2 | 4.6 | 4.1 | 4.1 | 383 (292) |
| 2 | 5.8 | 5.7 | 5.2 | 4.5 | 4.2 | 4.1 | 341 (269) |
| 3 | 5.8 | 5.7 | 5.2 | 4.7 | 4.2 | 4.2 | 226 (212) |
| 4 | 5.8 | 5.7 | 5.2 | 4.4 | 4.1 | 4.1 | 363 (288) |
| 5 | 5.8 | 5.7 | 5.2 | 4.5 | 4.1 | 4.1 | 401 (310) |
| 6 | 5.8 | 5.7 | 5.1 | 4.5 | 4.1 | 4.1 | 286 (241) |
| 7 | 5.8 | 5.7 | 5.4 | 4.6 | 4.2 | 4.1 | 434 (332) |
| 8 | 5.8 | 5.7 | 5.2 | 4.6 | 4.1 | 4.2 | 408 (345) |
| 9 | 5.8 | 5.7 | 5.2 | 4.5 | 4.1 | 4.1 | 332 (310) |
| 10 | 5.8 | 5.7 | 5.1 | 4.5 | 4.1 | 4.1 | 289 (229) |
| Av. | 5.8 | 5.7 | 5.2 | 4.5 | 4.1 | 4.1 | 336 (282) |

TABLE 12
MEDIUM D-1

| No. | 0 | 1 | 2 | 3 | 4 | 5 | Flies |
|---|---|---|---|---|---|---|---|
| 1 | 5.8 | 5.6 | 5.3 | 4.4 | 4.2 | 4.2 | 295 |
| 2 | 5.8 | 5.6 | 5.2 | 4.4 | 4.2 | 4.2 | 201 |
| 3 | 5.8 | 5.6 | 5.2 | 4.3 | 4.2 | 4.2 | 195 |
| 4 | 5.8 | 5.6 | 5.2 | 4.4 | 4.2 | 4.2 | 266 |
| 5 | 5.8 | 5.5 | 5.2 | 4.4 | 4.2 | 4.2 | 235 |
| 6 | 5.8 | 5.6 | 5.2 | 4.5 | 4.3 | 4.3 | 259 |
| 7 | 5.8 | 5.6 | 5.2 | 4.4 | 4.2 | 4.2 | 198 |
| 8 | 5.8 | 5.6 | 5.2 | 4.3 | 4.2 | 4.2 | 196 |
| 9 | 5.8 | 5.6 | 5.2 | 4.4 | 4.2 | 4.2 | 199 |
| 10 | 5.8 | 5.6 | 5.2 | 4.6 | 4.2 | 4.2 | 247 |
| Av. | 5.8 | 5.6 | 5.2 | 4.4 | 4.2 | 4.2 | 230 |

failed and all further flies were killed. The average for the eight days was 336, or of the nine normal cultures, 349. Since the egg laying was only 6 days instead of ten and the counting period eight days instead of ten, the average of 336 or, more accurately, 349, must be considered remarkably high. The best previous series (Medium C, Table 9) had given an average of 308 for ten and ten as periods for egg laying and emergence. In parentheses in the final column of Table 11 are given totals for the first six days of emergence, for comparison with series D-1 and D-2 which were cut short after six days' emergence, by the same incubator failure. The six days' average for ten cultures was 282, or 289 for the nine normal cultures.

In Table 12 are given the results of similar cultures, using medium D-1 (0.1 gm $KH_2PO_4$). The pH readings were little different from the plain D results. The emergence period was limited to six days by incubator failure. The average for all ten cultures was 230, or 233 for the nine cultures in which the female was alive and normal at the end of the six days' egg laying period (*i.e.*, excluding culture 7). This value of 233 is distinctly lower than the comparable value of 289 for plain medium C. Evidently the presence of the phosphate had not improved conditions here but rather the reverse.

TABLE 13
MEDIUM D-2

| No. | 0 | 1 | 2 | 3 | 4 | 5 | Flies |
|---|---|---|---|---|---|---|---|
| 1 | 5.8 | 4.9 | 4.7 | 4.5 | 4.2 | 4.1 | 186 |
| 2 | 5.8 | 5.2 | 4.8 | 4.5 | 4.2 | 4.0 | 194 |
| 3 | 5.8 | 5.2 | 4.8 | 4.5 | 4.2 | 4.1 | 126 |
| 4 | 5.8 | 4.9 | 4.7 | 4.5 | 4.3 | 4.1 | 276 |
| 5 | 5.8 | 5.2 | 4.7 | 4.5 | 4.3 | 4.1 | 167 |
| 6 | 5.8 | 5.0 | 4.8 | 4.5 | 4.2 | 4.1 | 122 |
| 7 | 5.8 | 4.9 | 4.8 | 4.5 | 4.2 | 4.1 | 276 |
| 8 | 5.8 | 5.0 | 4.7 | 4.5 | 4.1 | 4.0 | 354 |
| 9 | 5.8 | 5.0 | 4.6 | 4.5 | 4.2 | 4.0 | 87 |
| 10 | 5.8 | 5.2 | 4.7 | 4.6 | 4.3 | 4.1 | 282 |
| Av. | 5.8 | 5.1 | 4.7 | 4.5 | 4.2 | 4.1 | 206 |

TABLE 14
MEDIUM D-3

| No. | 0 | 1 | 2 | 3 | 4 | 5 | Flies |
|---|---|---|---|---|---|---|---|
| 1 | 5.8 | 5.7 | 5.5 | 4.8 | 4.8 | 4.8 | 347 (275) |
| 2 | 5.8 | 5.7 | 5.4 | 4.8 | 4.8 | 4.8 | 308 (265) |
| 3 | 5.8 | 5.7 | 5.4 | 4.8 | 4.8 | 4.8 | 292 (257) |
| 4 | 5.8 | 5.6 | 5.5 | 5.2 | 4.9 | 4.8 | 239 (212) |
| 5 | 5.8 | 5.7 | 5.4 | 5.0 | 5.0 | 4.9 | 397 (335) |
| 6 | 5.8 | 5.7 | 5.5 | 4.9 | 4.8 | 4.8 | 312 (251) |
| 7 | 5.8 | 5.7 | 5.4 | 4.8 | 4.8 | 4.8 | 366 (293) |
| 8 | 5.8 | 5.6 | 5.5 | 4.8 | 4.8 | 4.8 | 337 (302) |
| 9 | 5.8 | 5.8 | 5.5 | 4.9 | 4.9 | 4.8 | 367 (319) |
| 10 | 5.8 | 5.6 | 5.5 | 4.9 | 4.9 | 4.9 | 355 (299) |
| Av. | 5.8 | 5.7 | 5.5 | 4.9 | 4.8 | 4.8 | 332 (281) |

In Table 13 are given the results of tests of medium D-2, modified by the presence of ammonium tartrate. The pH readings were practically identical with those of the plain and of the phosphated media. The offspring were recorded for six days only because of the failure of the incubator on the seventh day. In cultures 5 and 6 the female died after 4 days of egg laying. The other cultures were from 6 days egg laying. The emergence of offspring was delayed one day in three cultures (4, 7 and 10); two days in one culture (3); and three days in another (9). The numbers of offspring fell off early in two cultures (5 and 6), and in these the mothers were known to have died early. The resulting progenies showed a range of from 87 to 354 offspring, with an average of 206 for all, or 221 for the eight normals, excluding 5 and 6. This average was even lower than that for the series raised on medium D-1, modified by $KH_2PO_4$, and there was far greater variability and unevenness in the results. A relatively low modal production and a high variability had likewise characterized the results of both previous tests of the effect of ammonium tartrate.

In Table 14 are given the results of a trial of medium D-3 with a richer nitrogen supply in the form of $KNO_3$. Here the pH readings changed the least of any observed, reaching a minimum of 4.8. The egg laying was for six days and the counting for eight days. Tests of D-3 were begun simultaneously with D and likewise of D-1 with D-2; all ended simultaneously because of the incubator failure. The female of culture 4 was injured in the taking of samples for pH determinations, and thereafter walked about sluggishly with the wings curled together above her back. Her low output (239 or 212) was presumably due to this injury. The other nine cultures gave an average of 342 flies for eight days' emergence and of 299 for six days' emergence. This record of 299 was slightly better than the comparable 289 for plain medium D, and the results were more uniform, ranging from 251 to 335 instead of from 229 to 345. The $KNO_3$ was thus

no detrimental factor but was possibly a slightly favorable modification.

### Routine Medium, 1928

With the failure of the incubator, the series of tests was broken. However, the two most important improvements, the elimination of $SO_2$ and other preservatives from the molasses and the rise in the percentage of agar, were incorporated in the routine procedure for making cultures for the stocks of Drosophila and for the experiments. The composition of the medium was further changed by lowering the content of the cornmeal and increasing that of molasses and water. These changes were for greater ease in preparing the media (pouring, etc.) and to reduce the granulation of the surface under the action of many larvae. The formula was as follows:

| | | |
|---|---|---|
| Medium 1928 | Water | 71.6 |
| | Cornmeal | 14.0 |
| | Molasses | 13.0 |
| | Agar | 1.4 |

### Determination of pH of Buffered Cultures by the Quinhydrone Electrode

With the above medium as a basis some experiments on buffering were made by Mr. Albert Tyler, to whom we are indebted for the following account.

The pH measurements were made with the quinhydrone electrode, which was preferable in that it was not poisoned by the media.

The amount of buffer to be used was determined by weighing out a sample of the media, taken from a 16-day-old culture, getting its E.M.F. in the set-up and then triturating in the buffer until the E.M.F. reached a value which corresponded with the pH of 4.0. Cultures were then made up, using the calculated amounts of acetate, phosphate, tartrate and citrate buffers. None of these gave offspring and the parents died. When amounts were used to give buffer action up to twelve days, the

same results were obtained. The amounts finally used corresponded to the acidity at the end of about six days.

In none of the sets of cultures was the output of flies greater than in the controls of plain medium 1928. In all there was greater loss of cultures due to death of parents and to heavier growth of molds. In some cultures to which acetic acid was added the few flies were all very small. The phosphated medium gave flies larger than normal but not more numerous than in the controls. The pH maintained the highest level, throughout the nineteen days of the determinations, in the cultures to which only sodium acetate had been added. The number of cultures (5) used in any one test was too small to give more than a rough indication of the situation.

### California Routine Medium

On account of the drier climate of California it was found advisable to still further increase the water content to withstand the greater evaporation. This was accompanied by a decrease in the proportionate amount of cornmeal and an increase in the total food to 60 cc per culture. The formula in routine use at the laboratories of the California Institute of Technology during the fall of 1928 and continuously to the present date is as follows:

| | | |
|---|---|---|
| California medium | Water | 75.0 |
| | Cornmeal (chicken feed) | 10.0 |
| | Molasses ("Grandma's") | 13.5 |
| | Agar | 1.5 |

In preparing this food the cornmeal is first soaked in enough of the water to wet it thoroughly and leave an excess of water after all the cornmeal is stirred in smoothly. The agar is then put to heating in about two thirds of the remaining water. As the water and agar come to a boil more of the water is added with stirring. This method of adding the water hastens the solution of the agar. After solution of the agar the molasses is stirred in and then the wet cornmeal. After the whole is brought to a boil and kept boiling for about seven

minutes the remaining water (about a tenth of the whole water) is stirred in. The medium is then ready to be poured into the sterilized half-pint culture bottles by means of a large funnel and hose controlled by a large spring clamp. The medium should be poured about one inch deep in the bottles. As low as three quarters of an inch will do, especially for stock cultures, but the greater depth will give a greater output and better ratios for weak classes of offspring. The medium should be made up in fairly large lots, 50 to 200 bottles. A reserve of bottles can be kept for two or three days in a cold room or refrigerator, though this is not advisable if bottles can be made fresh as required.

### Determination of pH by Block Comparator

The testing of the pH of the California routine medium was undertaken by Mr. N. K. Schaffer at the California Institute of Technology. He employed La Motte color standards and La Motte 0.4 per cent. standardized indicator solutions. Instead of the drop method employed by Darby, Schaffer used test-tubes and a block comparator, as described by Clark (1928).

In the tests carried out in March, 1929 (Table 15), the pH values differed from the previous findings for cornmeal molasses agar media only in the slower fall to the minimum pH of 3.7 in the case of cultures without flies and 3.6 for the cultures with flies. The output was low, 159 for six cultures that produced consistently, and 0, 5, 11 and 41 for four others. The failures and the low productivity do not constitute a fair representation of the food medium, since a wild stock was used (Oregon R) which is now known to behave erratically.

Four years' experience with this medium has shown that it is excellent, certainly more satisfactory than any previously used in routine work, and relatively inexpensive. Molds are practically unknown unless a culture has already failed from sterility. Molds may develop if cultures are kept very long. Hence it is advisable to

TABLE 15

California Routine Medium, 1929; Cultures 1–10, without Flies; 11–16, with

| No. | Days | | | | | | | | | | | | | | | | | | Flies |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| 1 | 5.6 | 5.5 | 4.8 | 4.2 | 4.0 | 3.8 | 3.7 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | ..... |
| 2 | 5.6 | 5.6 | 4.4 | 4.3 | 4.1 | 3.8 | 3.8 | 3.7 | 3.7 | 3.6 | 3.6 | 3.5 | 3.5 | 3.6 | 3.6 | 3.6 | 3.7 | 3.7 | 3.8 | ..... |
| 3 | 5.6 | 5.5 | 5.2 | 4.2 | 4.0 | 3.8 | 3.7 | 3.7 | 3.7 | 3.7 | 3.6 | 3.5 | 3.6 | 3.5 | 3.6 | 3.5 | 3.6 | 3.6 | 3.6 | ..... |
| 4 | 5.6 | 5.6 | 4.8 | 4.4 | 4.0 | 3.7 | 3.8 | 3.6 | 3.7 | 3.6 | 3.6 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.7 | 3.7 | 3.7 | ..... |
| 5 | 5.6 | 5.6 | 5.0 | 4.2 | 4.1 | 4.0 | 3.8 | 3.8 | 3.8 | 3.8 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | ..... |
| 6 | 5.6 | 5.0 | 4.6 | 4.0 | 4.0 | 4.0 | 3.8 | 3.8 | 3.7 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.8 | 3.7 | 3.6 | 3.8 | 3.8 | ..... |
| 7 | 5.6 | 5.6 | 4.8 | 4.1 | 4.0 | 3.8 | 3.7 | 3.7 | 3.7 | 3.7 | 3.6 | 3.6 | 3.7 | 3.6 | 3.7 | 3.6 | 3.6 | 3.6 | 3.8 | ..... |
| 8 | 5.6 | 5.4 | 4.4 | 4.2 | 4.0 | 3.8 | 3.7 | 3.7 | 3.7 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | ..... |
| 9 | 5.6 | 5.6 | 4.7 | 4.1 | 3.9 | 3.8 | 3.7 | 3.6 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.7 | 3.7 | 4.0 | 3.7 | 3.8 | 4.4 | ..... |
| 10 | 5.6 | 5.6 | 5.0 | 4.1 | 4.1 | 3.9 | 3.8 | 3.7 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.5 | 3.6 | 3.6 | 3.6 | ..... |
| Av. | 5.6 | 5.5 | 4.8 | 4.2 | 4.0 | 3.9 | 3.8 | 3.7 | 3.7 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.7 | 3.7 | 3.6 | 3.7 | 3.8 | ..... |
| 11 | 5.6 | 5.0 | 5.2 | 4.1 | 4.0 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.5 | 3.5 | 3.6 | 3.6 | 3.7 | 4.1 | 4.4 | 5.0 | 5.2 | 167 |
| 12 | 5.6 | 5.5 | 4.6 | 4.0 | 3.8 | 3.6 | 3.6 | 3.6 | 3.6 | 3.5 | 3.5 | 3.5 | 3.7 | 3.7 | 4.0 | 4.0 | 4.5 | 4.7 | 4.4 | 112 |
| 13 | 5.6 | 5.6 | 5.1 | 3.8 | 3.6 | 3.6 | 3.5 | 3.6 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.4 | 3.4 | 3.6 | 3.6 | 4.0 | 4.0 | 158 |
| 14 | 5.6 | 5.0 | 4.6 | 4.1 | 3.8 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.5 | 3.5 | 3.6 | 3.7 | 3.6 | 4.0 | 4.5 | 4.3 | 4.2 | 166 |
| 15 | 5.6 | 5.6 | 4.6 | 4.0 | 3.8 | 3.8 | 3.7 | 3.6 | 3.5 | 3.6 | 3.5 | 3.5 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 4.0 | 4.0 | 188 |
| 16 | 5.6 | 5.4 | 4.4 | 4.2 | 3.8 | 3.8 | 3.7 | 3.6 | 3.6 | 3.6 | 3.5 | 3.5 | 3.6 | 3.6 | 3.7 | 4.2 | 4.0 | 5.0 | 5.4 | 163 |
| Av. | 5.6 | 5.4 | 4.8 | 4.0 | 3.8 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 | 3.5 | 3.5 | 3.6 | 3.6 | 3.7 | 3.9 | 4.1 | 4.5 | 4.6 | 159 |

discard all cultures as soon as counts are finished or a culture is found to be sterile. Discarded cultures should be washed up at once and not allowed to stand about growing crops of molds (and perhaps also mites). This formula has been used rather extensively in other laboratories also, and while we have no detailed reports on it, it seems to have been acceptable.

### Stern's Formula

Dr. C. Stern was working in the Columbia Laboratory at the time of changing over from the banana agar to the cornmeal molasses agar method. In a recent publication (1929), Stern gives the following formula of the medium in current use in the Kaiser Wilhelm Institute.

| | | | | | |
|---|---|---|---|---|---|
| | Water | 2125 | | Water | 600 |
| A | Molasses | 375 | B | | |
| | Agar | 30 | | Cornmeal | 400 |

Boil mixture A until the agar has dissolved, then stir in mixture B and continue heating until thickening has occurred.

### Effect of pH on Pupation

After the foregoing researches were completed, one of the writers (Darby) began some researches on the pupation of *Anastrepha ludens* (Loew), the Mexican fruit fly, in the laboratory of the U. S. Bureau of Entomology in Mexico City. The larvae of this fly, when full grown, leave the fruit in which they have passed their larval life, burrow into the soil, going some distance until clear of the acids produced by the decaying fruits. Sometimes they are forced by circumstances to pupate within the fruit, and in these cases the pupal shell is exceedingly thin. The acidity of the fruit, guava in particular, drops to pH 3.0 and is therefore equal to the acidity of Pearl's cultures. This recalls the fact that in very acid bottles Drosophila larvae generally climb up the paper out of the acid medium and may even go as far as the stoppers. If the acidity were forced up to pH 3.0, as suggested by

Pearl, this factor would become important in the formation of viable pupae.

## SUMMARY AND CONCLUSIONS

A general survey of the results of the foregoing experiments on various cornmeal molasses agar media suggests the following conclusions: With respect to pH there was uniformly a very high similarity between the several cultures of a set. The different sets of cultures that were similar in formula and treatment, but made up at different times, showed definite but not very large differences in the initial pH, in the length of time taken to reach the minimum, in the value of this minimum and in the amount of subsequent rise.

All sets prepared from unbuffered media showed essentially the same type of curve—an initial pH value of about 5.8 (5.6–6.0) which was maintained for one or two days, and a rapid fall occupying one to three days, usually two, to a minimum of 3.6 to 3.8. This minimum was maintained about three days, and was followed by a gradual rise of slight amount (0.3 to 1.0), reaching a nearly uniform condition at a pH of 3.8 to 4.8. The presence of flies hastened the drop by about one day, possibly by scattering the yeast more thoroughly. The final rise in pH was greater in the cultures containing flies, probably on account of the larger amounts of alkaline waste products from the growing larvae. With unbuffered media the pH during the major portion of the course of the cultures lies below the optimum value for yeast, which is approximately 4.5.

In the media modified by the addition of chemicals having buffering action, the initial pH was most modified, and subsequently the cultures approached in value the controls. In two sets the pH did not drop to a minimum which was as low as elsewhere. In one of these the salt added was sodium acetate, which tends to buffer at a high pH (4.6 ±), relative to the normal minimum of unbuffered cultures (3.7 ±). In the other case, the salt added

was $NaNO_3$ and it is not clear why this should have kept the pH high, for $HNO_3$ is not a weakly ionized acid. Since the acidity present in the normal culture is due mainly to acetic acid, it would seem that the simplest and most natural buffer would be Na acetate, especially since it tends to buffer at a more favorable pH than the phosphate does.

With respect to the output of flies, the improvements made raised the output from the initial average of 151 (Table 6) to a high one of 342 (Table 14) for eight days' emergence, equivalent to about 425 for ten days' emergence. This improvement was due principally to removing the harmful $SO_2$, to increasing the proportion of agar (1.0 to 1.4) and to increasing the quantity of food (50 cc to 60 cc) per culture. Relatively little favorable effect is attributable to the chemicals added. Phosphate tended to produce larger flies and sometimes to increase their numbers; tartrate tended to increase the variability of the output, with occasional phenomenal outputs; $NaNO_3$ gave a slightly more favorable effect, but has not been adequately tested.

In view of the relative amounts of variability met with in the tests, it would seem that pH determination on five cultures of a series would give a close enough approximation; while for the fly outputs above twenty cultures should be used.

## Possibilities

It is clear that the following chemical modifiers of the medium offer favorable possibilities that may develop through further trials: sodium acetate to keep the pH from falling too low; potassium phosphate for a directly favorable effect upon the growth processes; an ammonium salt (possibly ammonium nitrate) as a means of increasing the relatively low nitrogen content of the media, and alcohol as a deterrent of other growths than yeast.

The use of cornmeal as a combined food and filler has the objection of being more granular than fibrous in nature, especially when combined with the high agar content that seems desirable. Possible substitutes that may be suggested for trial are: crushed wheat, preferably a gluten wheat; rolled oats, with flat broad plates instead of small round granules; malted barley, with the contained barley hulls; coarsely ground raisins; figs.

Gelatine instead of agar would offer a source of food as well as of mechanical stiffness. But since it is attacked by bacteria, careful tests would be needed to develop a successful method of using it.

Absorbent or toweling paper in small strips can be worked down into the surface of the food, but is considerable labor to prepare. Cotton waste, glass wool or asbestos fibers could be used more conveniently and be more thoroughly incorporated in the food mass. In the spring of 1930 cotton waste was tried and was found to give a very favorable effect upon the surface texture of the medium. The best method seems to be to use the cotton waste in addition to toweling paper. The toweling paper is a double thickness piece two inches wide by four long and is embedded edgewise in the hot food immediately after the pouring and pushed down vertically until its lower edge touches the bottom of the bottle. The cotton is put in after the medium has solidified and been yeasted. A triangular groove about a half inch on the side is cut at one edge of the food mass and extending to the bottom. This channel offers escape for the $CO_2$ produced by fermentation and keeps the food from being driven up and out of place. This channel is stuffed loosely with cotton waste, using a square-ended narrow-bladed blunt knife. Extra waste is punched into the surface in tufts. The larvae forage preferably about the waste and pupate in the part that rises above the surface. The waste tends to keep the surface from crumbling.

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# SHORTER ARTICLES AND DISCUSSION

## SINGLE OR MULTIPLE SEX-FACTORS

WHETHER sex is dependent upon a single pair of factors in the so-called sex-chromosomes, or whether its expression is to be regarded as the combined outcome of a number of factors located in the autosomes as well as in the sex chromosomes is a question now actively debated. As is well known, Goldschmidt, from his *Lymantria* work, and Winge, from his work with *Lebistes,* have brought forward strong reasons for taking the former view. On the other hand, the Drosophilists, mainly on the chromosomal conditions accompanying the phenomenon of intersexuality in *Drosophila,* are strongly inclined to the latter view. Quite recently the question has been ably discussed by Bridges.[1] The object of the present note is to offer a brief criticism on some of the evidence on which he takes his stand. In arguing for the multiplicity of sex factors he has made use of the very ingenious work of Dobzhansky and Schultz,[2] who showed that the grade of intersexuality in *Drosophila* was shifted in the female direction when the flies contained a fragment of an X-chromosome in addition to the usual complement. Further, they claimed that the longer the piece of X-chromosome introduced, the greater was the shift towards the female condition. This, of course, would be a very strong piece of evidence for a multiplicity of sex factors in the X-chromosome, but in going over the data I feel doubtful whether they can bear this interpretation. In the experiments six different types of fragmented X-chromosomes were made use of, and these may be shown schematically in the following figure based on Table 2 in the paper of Dobzhansky and Schultz.

It will be noticed that five of them have in common the "yellow" end of the fragmented X, while one (126) does not contain it. Of the five with the "yellow" end two (107 and 118) are apparently identical.[3] 112 is slightly shorter, while L.V.M. is considerably longer. We may now turn to the figures showing the influence of the six duplications in producing a tendency to femaleness as compared with the controls. This was estimated by subdividing the intersexes in both influenced

[1] "The Genetics of Sex in Drosophila," *Sex and Internal Secretions,* 1932.

[2] *Proc. Nat. Acad. Sc.,* xvii, 1931, pp. 513–518.

[3] In the text 118 is stated to be longer than 107, but in Table II (p. 515) they are mapped out as being similar.

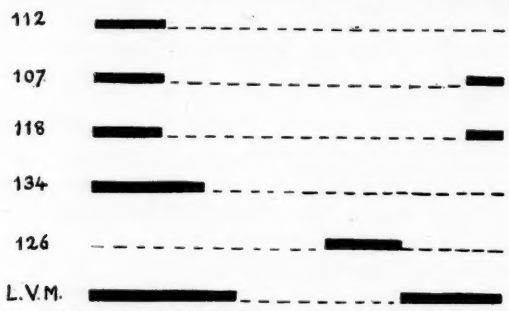


FIG. 1. Schematic representation of the 6 fragmented X-chromosomes. In every case the left end is that containing the locus for "yellow."

and controls into six grades, grade 6 being that nearest to the female type. Table 3 in the authors' paper gives the actual results, but for our purpose we need consider only the summary given in Table 4, which is here reproduced.

TABLE 4

THE MEAN TYPE OF THE INTERSEXES CARRYING THE DUPLICATION AND FREE FROM IT

| Duplication | Carrying the duplication | Control | Difference |
|---|---|---|---|
| 126 ..... | 2.74 ± 0.12 | 2.38 ± 0.21 | 0.36 ± 0.24 |
| 112 ..... | 4.75 ± 0.055 | 2.59 ± 0.08 | 1.56 ± 0.10 |
| 107 ..... | 4.43 ± 0.04 | 2.43 ± 0.06 | 2.00 ± 0.07 |
| 118 ..... | 4.53 ± 0.05 | 1.97 ± 0.075 | 2.56 ± 0.09 |
| 134 ..... | 4.70 ± 0.07 | 2.50 ± 0.06 | 2.20 ± 0.09 |
| L. V. M. | 6.0 | 2.6 | 3.4 |

In criticizing the data the points to which I wish to draw attention are as follows:

(1) For 126 the difference between the mean grade of the intersexes carrying the duplication (= 2.74) and that of the controls (= 2.38) is only 0.36—a difference less than twice the probable error, and therefore probably not significant. The result is compatible with the supposition that 126 exerts *no* influence on the grade of intersexuality. As already noted, 126 is the only duplication that does not carry the "yellow" end of the X-chromosome. On the other hand, all the other five duplications show a decided influence in shifting the grade of intersexuality towards the female direction. This is compatible with the supposition that a definite female sex factor occurs somewhere in

the "yellow" end of the X-chromosome, and not in the fragmented portion carried by 126.

(2) We may next consider the contention of the authors that the amount of shifting is proportional to the length of the X fragment, and in doing so we shall take account only of the four duplications 112, 107, 118 and 134. For L. V. M. obviously stands apart from the rest. The figures in column 1 of Table 4 show a gradually increasing shift for these four duplications when arranged in order of increased size. Nevertheless, the extremes are separated by .55 of a grade only, and the difference between 107 and 118 is barely significant. When, however, we turn to the controls in column 2 we meet with marked irregularity, the extremes being separated by .64 of a grade. Now if the triploid mothers used were of the same stock, and there is nothing in the account to lead us to suppose otherwise, this variability may well make us hesitate before accepting the apparently orderly increase shown in the figures of column 1. For it may be merely accidental. If on the other hand we are to regard the control figures as in each case an accurate measure of the material upon which the fragmented X works, and to take the figures in column 3 as the significant ones, then the data do not quite bear out the authors' contention. For the effect of the longest fragment 134 is less than that of 118, and shows a barely significant difference from that of 107.

As already noted, the case of L. V. M. is obviously distinct, and it seems reasonable to suppose that some modifying factor is here to be found in this longest fragment which is not present in the others.

And here a brief word on what the authors term "mere modifying factors." They take the view that since such factors show the same quantitative relations as the sex factors proper the distinction between the two classes is an arbitrary one at best, wherein they are opposed to Goldschmidt and others. Over this question of modifiers I incline to think that the two parties are a little at cross purposes. The "modifiers," among whom I would count myself, appear to have this at any rate in common, *viz.*, the view that the modifying factor has no influence on the phenotypic character associated with the factor modified unless this factor be present. There are many modifying factors for shades of flower color, but in so far as color is concerned they exert no influence unless the factor for color itself be present. So with sex. The "modifiers" would say that there is a definite

sex factor[5] capable of producing a definite effect of itself, but its expression can be influenced by certain modifying factors. In the absence of the sex factor the modifiers would not of themselves produce any effect in so far as the expression of sex is concerned. On the other hand, I gather that in the conception of the Drosophilists sex is the outcome of a number of factors and that no single one of these factors is absolutely essential; though of course if the complement be abnormal the expression of the character will be abnormal also. In this sense there does seem to me to be a real difference between the Drosophilist's conception of sex and that of his opponents. Whether the two can be brought into line, or whether there is a fundamental difference between *Drosophila* and the other creatures hitherto investigated must be left for future work to decide.

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## INTERLOCKING AS A DEMONSTRATION OF THE OCCURRENCE OF GENETICAL CROSSING-OVER DURING CHIASMA FORMATION

DURING an examination of chromosome behavior at meiosis in *Lilium regale* a very interesting and exceptional case of bivalent interlocking was observed at diakinesis. One of the bivalents concerned had a loop interlocked with two loops of the other, each side of the loop of the first bivalent passing through one loop of the second.

Figs. 1–6 are untouched photomicrographs of the configuration, taken at gradually descending foci; Fig. 3 being at the critical focus and showing the center chiasma of the second (horizontal) bivalent between the sides of a loop of the first (vertical) bivalent.

At zygotene the constituent chromosomes of the second bivalent must have become paired between the chromosomes of the first one, so giving when pairing was completed a configuration as illustrated in the diagram (Fig. 7). Chiasmata subsequently formed at the points indicated, and one of the end ones of the second bivalent terminalized, so giving rise to the observed diakinesis configuration.

This example of interlocking has an important bearing on the relations between chiasma-formation and crossing-over. If

[5] Or pair of factors.

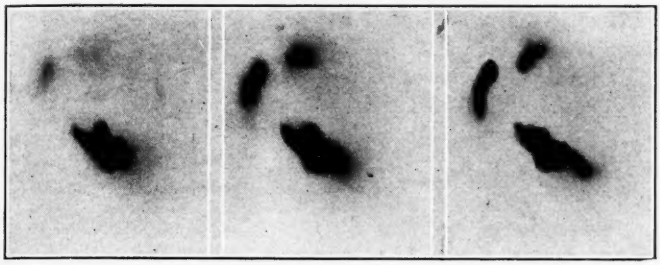


FIG. 1 FIG. 2 FIG. 3

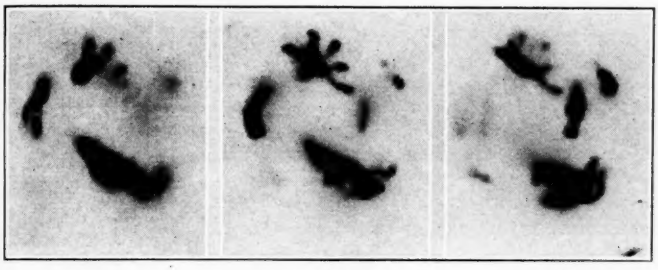


FIG. 4 FIG. 5 FIG. 6

FIGS. 1–6. Photomicrographs of the two interlocked bivalents taken at descending foci; Fig. 3 shows the critical central chiasma with the interlocked loop of the other bivalent enclosing it. (Photos taken by Mr. H. C. Osterstock.) × ca 1400.

chiasmata arise by the opening out of reductional and equational loops as the "classical" theory postulates, in one loop of the second bivalent identical chromatids would be on opposite sides of one chromosome (pair of chromatids) of the first bivalent (see Fig. 8). This is absurd, as bivalent interlocking must be

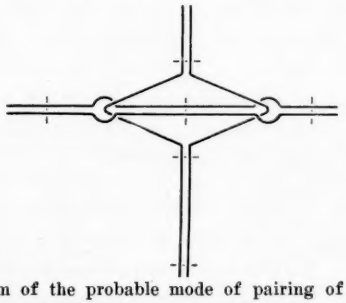
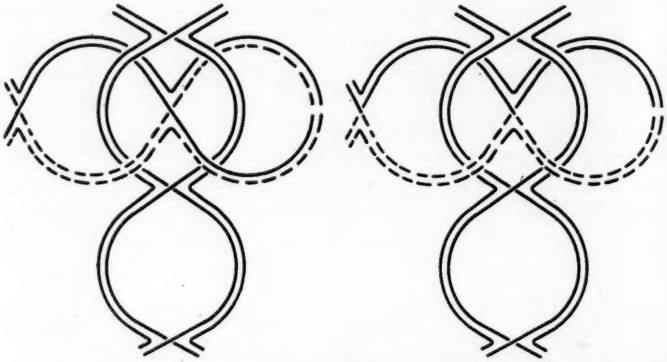


FIG. 7. Diagram of the probable mode of pairing of the four chromosomes at pachytene. The broken transverse lines indicate the places where chiasmata subsequently arose.



FIGS. 8 and 9. Line diagrams of the observed diakinesis configurations. Fig. 8 shows the interpretation on the "classical" theory, leading to identical chromatids being on opposite sides of one interlocked chromosome—an impossibility. Fig. 9 shows the interpretation on the chiasmatype theory.

determined before chiasma formation, *i.e.*, at early prophase before the split of the primary chromosomes into chromatids. Therefore, the center chiasma of the second bivalent must have arisen by genetical crossing-over between two of the four chromatids of this bivalent (see Fig. 9). Thus this case of interlocking is a cytological demonstration of genetical crossing-over at the critical chiasma.

It must be noted that this configuration could have arisen by the opening out of reductional and equational loops if there had been, originally, two chiasmata in the second bivalent between the sides of the interlocked loop of the other bivalent, one of these chiasmata subsequently breaking. Sax (1932) has made a similar postulation with regard to Darlington's demonstration of crossing-over in quadrivalents of tetrasomic *Hyacinthus*. Now in *Lilium*, as in *Hyacinthus*, the reduction in the number of chiasmata from diplotene to metaphase is very small, and so such breakage of a chiasma would be improbable, unless interlocking itself increased chiasma breaking, a postulation which disagrees with the observations of Gairdner and Darlington (1931) on *Campanula persicifolia* and the present author (unpublished) on *Lilium* species. Consequently, it is improbable that such a formation of two chiasmata followed by a breakage of one of them has given rise to the observed configuration.

Hence the exceptional case of interlocking reported here very

strongly supports the chiasmatype theory, as restated by Darlington (1931): "(i) A chiasma is constituted by genetical crossing-over between two of the four chromatids taking part in it, or (ii) association at diplotene is between chromatids derived from the same somatic chromosome."

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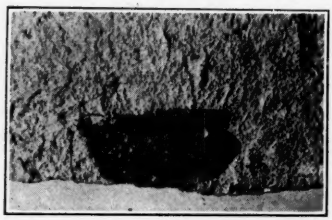
## A SECOND MOTH FROM THE COLORADO EOCENE

IN the AMERICAN NATURALIST, lxv (1931), p. 354, the fossil moth, *Chionaemopsis quadrifasciatus* Cockerell and LeVeque, was described from the Colorado Eocene, being apparently the oldest known lepidopterous insect. In the same volume (p. 479), Professor W. T. M. Forbes calls attention to the resemblance of this insect to the genus *Atteva*, belonging to a group apparently connecting the Yponomeutidae and the Pyralids.

Great interest was aroused recently, when Miss Mary L. Moore brought in a second fossil moth from rocks of the same age, the Green River Eocene. It was found by her brother, James Moore, near Rifle, Colorado. In the same collection is an excellent specimen of the previously described *Thamnotettix packardi* Cockerell.

The new specimen represents an entirely different genus and species, and shows the head, palpi and antennae, as well as both wings. It was preserved almost undamaged, the wings still covered with scales and the fringes almost perfect. My first impression was that it was an ordinary Pyralid, perhaps even to be referred to the genus Pyrausta; but the venation of the hind wing seems to indicate a member of the family Thyrididae, said by Hampson (1897) to be "closely allied to the ancestral stock of the Pyralidae." The ascertainable characters are as follows:



*Hexerites* NEW GENUS

Small *Pyrausta*-like moths with simple antennae; moderate, porrect, palpi; anterior wings rather narrow, the apical angle greater than a right angle, the tip obtuse; hind wings with the costa very straight, the apical angle rounded, but abrupt and less than a right angle.

Resembles the living (North American) genus *Hexeris* Grote, but the palpi are shorter; the anterior wings are more obtuse, less falcate at end; the outer margin of the hind wings is not at all concave. There is no trace of the characteristic fine lines or bands crossing the wings of *Hexeris*.

*Hexerites primalis* NEW SPECIES

Palpi rather slender, about 290 μ long; antennae simple, fairly stout, about 65 μ diameter; width of head 960 μ; anterior wing 6 mm long, lower margin 5 mm, outer margin 2.6 mm; costa straight for over 4 mm, then arched, curved; outer margin quite straight for nearly 2 mm below apex; venation not visible, owing to the heavy scaling; wing uniform light brown, without markings, except that the costa is very narrowly darkened, and the fringe on outer margin is slightly darkened. Hind wing visible in part only, where it appears above the fore wing; it is about 5.3 mm long, pale, with about the apical 2 mm lightly infuscated; whether the base is darkened can not be seen; vein Sc + Rl free at least 2160 μ from its end, where it is slightly curved upward; how much more is free can not be seen because the base is covered by anterior wing; veins Rs and Ml far apart, Rs going to apex of wing. It is the character of Sc + Rl of hind wings which appears to indicate a species of Thyrididae, and in this family the nearest relative appears to be *Hexeris*. I am greatly indebted to my colleague, Mr. Hugo Rodeck, for the photograph. The specimen is in the University of Colorado Museum.

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